LIVER INJURY

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TABLL OГ CONTENTS

Tenth Conference on Liver Injury

JOSIAH MACY, JR. FOUNDATION CONFERENCE PROGRAM

As an introduction to these Transactions of the Tenth Conference on Liver Injury, I would like to outline what it is that the Foundation hopes to accomplish by its Conference Program. We are interested, first of all, in furthering knowledge about liver injury and to this end the participants were brought together to exchange ideas, experiences, data, and methods. In addition to this particular goal, however, there is a further and perhaps more fundamental aim which is shared by all our conference groups: the promotion of meaningful communication between scientific disciplines.

The problem of communication between disciplines we feel to be a very real and a very urgent one, the most effective advancement of the whole of science being to a large extent dependent upon it. Because of the accelerating rate at which new knowledge is accumulating and because discoveries in one field so often result from information gained in quite another, channels must be established for the most relevant dissemination of this knowledge.

The increasing realization that nature itself recognizes no boundaries makes it evident also that the continued isolation of the several branches of science is a serious obstacle to scientific progress. Particularly is it so in medicine that the limited view through the lens of one discipline is no longer enough. For example, today medicine must be well versed in nuclear physics because of the tracer techniques and the injury which can result from radiation. At the other extreme, medicine is certainly a social science and, through mental health, must be concerned with economic and social questions. The answer, then, is not further fragmentation into increasingly isolated specialties, disciplines, and departments, but the integration of science and scientific knowledge for the enrichment of all branches. This integration, we feel, can be encouraged by providing opportunities for a multiprofessional approach to given topics.

Although the fertility of the multidiscipline approach is recognized, adequate provision is not made for it by our universities, scientific societies, and journals. And perhaps the presence of other hindering factors must be admitted. Partly semantic in nature, they

may also to some degree be psychological. Admittedly, it is oftentimes difficult to accept data derived from methods with which one is unfamiliar. By making free and informal discussion the central core of our meetings, we hope to achieve an atmosphere which minimizes as much as possible these emotional barriers.

Thus our meetings are in contrast to the usual scientific gatherings. They are not designed to present neat solutions to tidy problems but to elicit provocative discussion of the difficulties which are being encountered in research and practice. For this reason, we ask that the presentations be relatively brief and that emphasis be placed on discussion as the heart of the meeting. Our hope is that the participants will come prepared not to defend a single point of view but to take advantage of the meeting as an opportunity to speak with representatives of other disciplines in much the same way that they would talk with their own colleagues in their own laboratories.

We have, now, thirteen groups functioning under the Conference Program. The following topics are covered: adrenal cortex, aging, biological antioxidants, blood clotting, blood pressure, connective tissues, consciousness, cybernetics, infancy and childhood, liver injury, metabolic interrelations, nerve impulse, renal function, cold injury, and shock and circulatory homeostasis. When a new conference is organized, the Chairman, in consultation with the Foundation, selects fifteen scientists to be the nucleus of the group, and every effort is made to include representatives from all pertinent disciplines. From time to time new members are added by the group to fill gaps in viewpoint or technique. A limited number of guests are invited to attend each meeting but, for the purpose of promoting full participation by all members and guests, attendance at any meeting is limited to twenty-five. It is inevitable that in no topic can we possibly include more than a small fraction of the key investigators in the field, and one of the difficulties in forming a group like this is that it is necessary to leave out so many people whom we would like to include.

The transactions of these meetings are recorded and published. This is done because the Foundation wishes to make current thinking in a field available to all those working in it, and in addition, because it believes that conveying to those in other fields concerned with science, for example government officials, administrators, etc., the essential nature of scientific research is also an important problem in communication. Logic is a vital aspect of

science, but equally essential is the intuitive or creative aspect Research is as creative as the painting of a portrait or the composing of a symphony Although logic is, of course necessary in order to rearrange, to test, and to validate, research thrives on creativity which has its source in unconscious, nonrational processes Unfortunately, however, in the finished products which are presented to the world through our research reports this integral part of scientific endeavor is shriveled by the cold white light of logic By preserving the informality of our conferences in the published transactions, we hope to give a truer picture of what actually goes on in the minds of scientists and of the role which creativity plays

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INTRODUCTORY REMARKS

Fremont-Smith If you will permit me, I would like to make a few informal remarks to explain my feeling about a conference such as this Involved here, as at all meetings, is the problem of communication Each one of you has knowledge of a great mass of information, much of which would be exciting to the others I urge that you not be too selective nor too modest in expressing it for your comment may be intensely stimulating to someone else, and in turn the comments of others may reawaken in you knowledge which has been forgotten, buried, or discarded, new light may be thrown on a problem of your own In a situation as informal as this, there can be tremendously exciting results from what Dr Walter B Cannon in *The Way Of An Investigator** calls "the fertility of aggregation"

Another advantage of this type of meeting and one I hope you will make use of, is the opportunity to challenge someone else's statements The problem of semantics is so closely related to the problem of communication We sometimes find that people use the same word to mean different things, and this is your chance to go after a person and try to find out what it is that he really means, to make him be specific The phrase "with respect to what? is a convenient one indeed, I have found, when someone makes a statement that you wish to challenge

Then there are the emotionally induced blind spots which scientists being human, are no freer from than anybody else Blind spots for material coming from a certain laboratory or from a certain man are not rare Some people may have blind spots for material which is measured in a certain way There are the worshippers of the milligram, and the millimeter worshippers, and they will not understand each other I have exaggerated, of course, but bringing people together in continuing contact over a period of years may help them to understand each other I like Dale Carnegie's title *How to Win Friends and Influence People* for it illustrates that perhaps the only way to influence people is by first making friends with them

Some of you may know the book I was speaking of by Dr Cannon There is a delightful chapter in it called *The Value of the Hunch*,

* New York W W Norton & Co 1945

dealing with the unexpected flash of insight which suddenly comes to consciousness How many scientific discoveries have come in this way! Darwin in his autobiography describes how all the enormous mass of data which he had gathered had no particular meaning for him until, unexpectedly, while driving along a road, the concept of evolution came to him

I myself have been sat upon by my elders when I would tell them what I thought was a bright idea They'd say, 'Oh, but that is dangerous speculation!' Later I found that the dictionary says to speculate means to think upon or ponder about If that is dangerous, I am all for dangerous thinking! One of the main purposes of this meeting is to encourage you all to live dangerously by bringing forward speculation We shall perhaps criticize your off the cuff thinking but we shall be the better for having had the benefit of it And another attitude we have in these meetings is expressed by this phrase Don't speak when I am interrupting" We hope there will be lots of interrupting

Quoting again from Dr Cannon Horace Walpole writing to Horace Mann in 1754 proposed a new word 'serendipity' He got the idea from a fairy tale entitled *The Three Princes of Serendip* These princes were famous for a remarkable faculty As they traveled about their world, they kept discovering unexpectedly, quite useful things but things for which they were not seeking So serendipity applied to research would be taking advantage of an unexpected finding or of seeing something old in a new light Well I turn the meeting over now to your Chairman in the hope that serendipity will take place here

Best The duties of a Chairman are extremely easy at this meeting It is, as usual, informal, and we have kept the program to a minimum of headings so that we may explore thoroughly in the discussion whatever facets of the subject prove of most interest to the group

And now we will carry on with Dr Roy Turner on Serum Proteins and Lipids"

SECTION I

SERUM PROTEINS AND LIPIDS

ROY H TURNER

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I SENT MOST of you a copy of the manuscript which represents the first detailed publication of our group in New Orleans concerning work which has been in progress for more than three years(1) We have worked out a technique described in that paper and have made observations on a broad front All of our studies are inter related so publication has been very slow My greatest difficulty here this morning is that I must devote a good deal of time to description of results which I wish had been published and made available to you so that we could get around promptly to the most important opportunity offered by this Conference and that is to argue about interpretation I would like to clear my associates in advance of blame for any interpretations presented by me today which are proved later to be stupid because I am going to follow Dr Fremont Smith s advice and give you some hypotheses which in some instances have had little time to mature

I would like to point out to Dr Fremont Smith that serendipity has its embarrassing aspects This study of ours is an illustration of such embarrassment We started this work in an effort to expand some observations by Dr Hanger and his associates(2) using ultracentrifugation of whole serum with a view to better under standing of the mechanism of the thymol turbidity and the Hanger flocculation test Dr Kunkel has since then published many of the sort of findings that we made(3) We have not as yet published our results We added quantitative estimations of lipids to our procedure and soon became convinced that the quantity ultra centrifuge used as we were employing it had important possibili ties in determining the structure and eventually the function of lipid complexes or lipoproteins Our report today will be chiefly concerned with lipids because our information about lipids has run

* This work done in part under a contract from the Office of the Surgeon General U S Army and under the sponsorship of the Commission on Liver Disease Armed Forces Epidemiological Board

far ahead of our information about proteins We hope to catch up on proteins later The embarrassing aspect of this business is that it has brought up the necessity of constructing hypotheses of lipid transport I think it will become quite apparent if you are not already aware of it how exceedingly complex and difficult an undertaking it is and it is one which we had no desire to begin I should like very much to have the help of this group of experts in that aspect of our problem These sound like words of a timid soul and I will have to admit that there are terrifying aspects to the responsibility we have

As you who have seen our first paper know we use the quantity ultracentrifuge with whole serum — undiluted untreated whole serum — and get redistribution of proteins and lipids by high centrifugal forces In any study of the sort we are attempting the ideal is to get separation of components which are completely homogeneous We make no claim that we have accomplished this We doubt if any of our samples are completely homogeneous To certain tests some of our samples stand up very well as to homogeneity but for systems of this sort I think one should demand many different tests before saying that any sample is completely homogeneous Our emphasis is on quantitative chemical analysis and we have evidence of a high degree of chemical homogeneity in some of our material We believe that some of the methods of mathematical study of our data make it possible to describe some of the complexes at different levels even when these complexes are mixed with others Our general approach has been to determine the structure of lipid and protein aggregates or complexes and then we hope to determine their function We have tried to see how these aggregates change in concentration and perhaps composition under the stress of disease and under dietary stress The disease which we have studied most actively is acute hepatitis presumably acute virus hepatitis The dietary stress which we have best studied is starvation in normal individuals — the most enlightening experiments were five days in duration We have also observed the immediate effects of taking a lipid rich meal

We believe our procedure has three advantages The manipulation of proteins and lipids which brings about differentiation is gentle and the chance of denaturing them is less than some of the other procedures in use The description of samples we take from the ten levels in the column of centrifugate is by multiple methods and finally our data give an integrated picture of the proteins and lipids of the whole serum

May I turn aside at this point to say that our work proceeds v slowly It is a tedious, expensive, difficult procedure For e serum we study we make quantitative estimations for ele samples, the whole serum and the ten fractions of centrifug And because some of the chemical procedures are time-consum we go very slowly We have had only the part-time use of centrifuge

I would like to review briefly a description of normal serum our procedure as described in the manuscript with which you h been supplied(1) We describe in this paper the principal feat according to five zones of increasing density beginning at the The first zone, which includes the cream layer, we call the z of minimum density This zone on an average has about half neutral fat in the serum principally in the form of the so ca chylomicrons The second zone from the top is the zone of minim concentration, a water clear zone of inconstant composition the usual technique of centrifugation After prolonged centrifug this zone is widened and contains a fairly constant but low con tration of neutral fat and phospholipids The third zone from top is a broad zone which contains all four of the lipid compon we measure in considerable concentration and no doubt al special concentration of globulin as was shown in Figure 1, w is probably the protein portion of the lipoprotein in this zon low-medium density This zone contains about one half the t lipids of the whole serum with only a relatively small propor of neutral fat (correlated lipids) Albumin is the principal pr and β globulin the principal globulin although alpha globul present Immediately beneath the zone of low-medium de and joining it at a level where the centrifugate has a spe gravity about equal that of whole serum is the zone of high med density, which is peculiar in having low concentrations of cholesterol, but relatively high concentrations of cholesterol e The zone of high density at the bottom contains extremely concentrations of proteins especially globulins, and the only l present in significant concentration are phospholipids (uncorrel phospholipids) and neutral fat

We believe an important mechanism in effecting the distrib of lipids depends upon establishment of a density gradien sedimentation of heavier proteins albumin playing a large Such a density gradient determines whether certain of these complexes float or whether they sink whether some may float

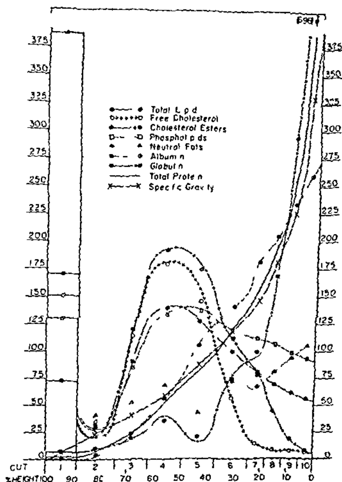


FIGURE 1 Mean distribution of proteins and lipids in centrifugate of untreated ... ed as per cent of ... res 1 to 10 along ... permission from ... press)

lower levels and others sink from higher levels so that they tend to reach their own density level We have good evidence that a fraction does not accurately settle at its own density level This fraction is larger toward the bottom of the column At the time we stop centrifugation the density gradient is still changing and therefore no absolute realignment of lipoprotein in terms of density of the surrounding medium is achieved

I wonder whether there are any questions at this point

Bollman Does it help any if you dilute the serum with a solvent of fixed density and thus minimize the effects of the presence of one protein on the separation of another?

Turner We have not explored that possibility One effect of dilution is to diminish the concentration of the compounds we are estimating, and that is one of our limiting factors For example, in estimations of neutral fat the error is increased when concentration is low We have had some experience in adding sucrose and saline so as to change the solvent density, but in the main we have stuck to our original idea of centrifuging unchanged whole serum

I should like to go now to a better description of the relationship that exists among lipids in the serum of healthy young men In each of the top five samples of centrifugate of normal serum, which include the three top zones, minimum density, minimum concentration and low-medium density, the relationship among certain lipids may be expressed by mathematical formulae This study will be reported in detail elsewhere(4) As an illustration, in the top zone, if the concentration of any two lipid components, such as free cholesterol and phospholipids are plotted against each other, a high degree of correlation is found and the relationship may be expressed by the formula for the line of regression The same thing may be said of the relationship between free cholesterol and cholesterol esters and neutral fat and free cholesterol Except for the two top samples, neutral fat does not correlate significantly with any other lipid component This may be in part due to the fact that in most of the samples we are considering, the concentration of neutral fat is of such an order as to cause considerable error in estimation, while in the top layer the values are in a range which gives a much smaller error On the other hand, it may mean that neutral fat is related to the lipoprotein complex by a different type of bond than other lipids In the top five samples there is high degree of correlation between the two forms of cholesterol and between either of them and phospholipids In the top sample there is one half as much esterified cholesterol per unit of free as in lower samples

All our descriptive data for the centrifugate in the top zone seem to be compatible with the concept that the lipids there consist mainly of globules of neutral fat contained in envelopes of lipoprotein This concept requires further examination and more accurate statement

Especially important is the evidence that a lipoprotein of a given class may differ in density We believe that density of lipid complex and specific gravity of centrifugate are closely related but not necessarily identical If this relationship is as close as it now appears there is continuous variation in density of a given sort of lipid complex throughout its distribution in the column of centrifugate The chemical analyses for lipids in contrast with density characteristics show such a high degree of constancy in certain interrelationships among three lipid components as to indicate *distribution of complexes containing lipids in the same proportions* over a wide range of specific gravity of centrifugate

We believed the question of density of lipoproteins at different *portions of the column was of such crucial importance to the* interpretation of our data that we developed two procedures which would give more exact information about density of lipid components and at the same time keep the identity of our complexes in terms of chemical analyses In one procedure we took samples of centrifugate and added them to serial concentrations of sucrose so chosen as to give sinking of lipids in some and floating in others so we call this technique the "sink float technique After recentrifugation we did complete lipid analyses on the top and bottom samples each of which equals 25% of volume and on the remaining middle sample In such experiments it is possible to relate accurately the density of the complex to that of the suspending medium We interpret results of such examinations as showing 1 That there is a close and systematic relationship between the density of the lipid complexes and that of the centrifugate in which they are found as far as the top one half of the column is concerned 2 *Study of a given sample of centrifugate taken from the* upper part of the low medium density zone shows the two forms of cholesterol and phospholipid in both normal and hepatitis centrifugate and neutral fat as well in hepatitis centrifugate as having exactly the same density characteristics by this test In the other procedure samples three and six of normal centrifugate were separately mixed with albumin solution so as to give a specific gravity of each mixture of 1 028 and then recentrifuged sampled and analyzed as in our standard procedure The free cholesterol peaks in these two mixtures were found at different specific gravity levels indicating clearly that the *molecules containing free choles*terol in sample six were heavier than those of sample four The wide distribution of this complex in centrifugate of whole serum is partly explained by variation in density of the complex and

partly by incomplete alignment with density of suspending medium These observations will be reported in detail elsewhere

In an effort to separate lipid concentrations into significant sub divisions we have employed correlation spot-graphs such as those shown in Figure 2 Graphs such as these were employed by Albrink, Man and Peters(5) in the study of the relationship between free cholesterol and lipid phosphorus in whole serum and they noted that the line of regression at zero free cholesterol cut the lipid phosphorus coordinate at 3 7 mgs %, a finding which we have con firmed Because none of their plotted points was near zero value these workers minimized the importance of this intercept In several of our graphs there is a sufficient number of points representing low values for free cholesterol to establish beyond doubt the fact that the line of regression does intercept the zero free cholesterol coordinate at a point far removed from the origin Interpretation of these intercept values in terms of concentration of specific lipid complexes is a more hazardous step, but it is a step which we feel impelled to take We use this intercept value

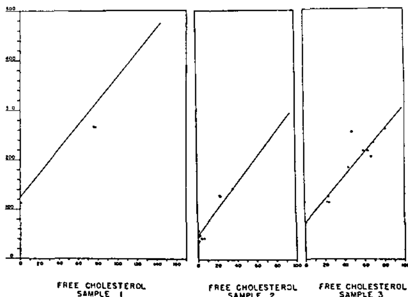


FIGURE 2 Three spot graphs showing the relationship between concentrations of free cholesterol and phospholipids in 3 top samples of centrifugate of sera of 18 normal individuals Data from graphs of this type were used in separation of phospholipids into two fractions correlated and uncorrelated

as an index of the quantity of lipid unrelated mathematically to free cholesterol

We interpret such graphs as these as showing the presence of two sorts of lipid complexes 1) *Correlated* * This complex or group of complexes contains at least 3 lipid components and has the distribution in the column of free cholesterol In other words concentrations of free cholesterol cholesterol esters and phospholipids are highly correlated This complex appears to contain all the free cholesterol of the serum excepting that found in the cream layer and the possibility exists that it is this lipoprotein or a slight modification thereof which makes up the envelope of the globules of neutral fat in the cream layer where the organization of lipids demonstrated by correlation methods seems to differ chiefly in two respects high concentration of neutral fat and one half the esters per unit of free cholesterol 2) *Uncorrelated phospholipids* A complex which may contain no other lipid than phospholipid although there is evidence that neutral fat may be a constituent These two lipids only are found at the bottom of the column Likewise after *prolonged centrifugation it is these two lipids which are found in* the broad water clear zone of minimum concentration Perhaps this finding of phospholipids and neutral fat as the only lipids at the extreme ends of the column of centrifugate represents extremes in variation in molecular structure of the uncorrelated phospholipid-containing lipoprotein the lightest and smallest molecules near the top and the heaviest and largest toward the bottom This fraction of phospholipid in the top five samples is identifiable only by the aid of correlation analysis the concentration there is assumed to be that indicated by the intercept value on the correlation graphs Phospholipid of this sort is uncorrelated with and presumably unbound to free cholesterol 3) *Uncorrelated esters* This complex depends for its identity upon the same sort of evidence as the uncorrelated phospholipids There are two peaks of concentration in normal centrifugate

In graphs in Figures 3 and 4 are shown normal mean distribution of the two kinds of complexes each for phospholipids and cholesterol esters the correlated and uncorrelated *All these lipid* complexes show wide distribution in the column of centrifugate By this method of study we suggest kinship of complexes of phospholipids in the two zones of highest density with some in centrifugate of lower density There seem to be two concentrations of

* Unsatisfactory terminology but the best we have thought of so far —*R H Turner*

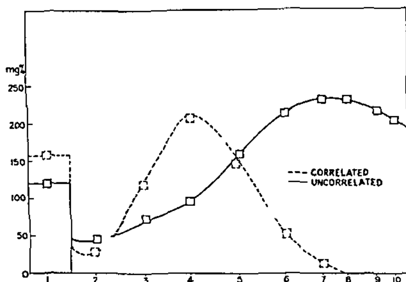


FIGURE 3 Graph shows mean distribution of phospholipids of two kinds as distinguished by mathematical analysis of lipid values of centrifugate of normal serum That which is correlated with free cholesterol and the uncorrelated Plotted points of uncorrelated phospholipid represent concentration where the line of regres

complexes containing cholesterol esters without free cholesterol that of higher density with a peak in centrifugate of specific gravity of 1 030 which gives distinctive character to the zone of high medium density and a second which gives a peak at 1 012 specific gravity

One of the most puzzling aspects of interpretation is the apparent conflict between homogeneity of lipid composition and inhomogeneity of particle density of lipoproteins as defined above There *are two main possibilities for explaining such variation of density* associated with constancy of lipid composition 1) It is attributable to lipid composition not shown in our analyses Neutral fat must be considered as a possible constituent of such complexes, but there is no general evidence of negative correlation between concentration

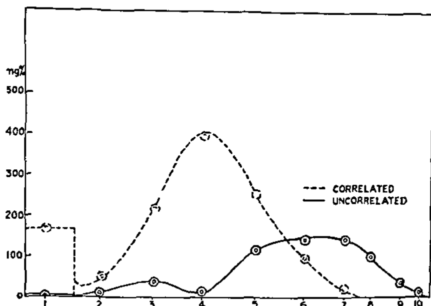


FIGURE 4 Graph shows mean distribution of cholesterol esters of two kinds as distinguished by mathematical analysis of lipid values of centrifugate of normal serum That which is correlated with free cholesterol and the uncorrelated Method of constructing this graph similar to that used for Figure 3 and explained in that legend This graph indicates a mean of 70 per cent of esters in normal serum are associated with free cholesterol

of this low density component and specific gravity of centrifugate at different levels The less dense of the two ester complexes may contain a high proportion of neutral fat *in some normal serum* and especially in hepatitis One might imagine some sort of physical union of two lipoproteins in varying proportions such as the two complexes of different densities containing phospholipids 2) The quantitative relationship between the protein and lipid portions of the lipoprotein molecule is the variable which determines density Such a concept multiplies the number of kinds of lipoproteins and requires that each of our classes be considered a group I believe from study of our data that two trial concepts of lipid transport should be introduced at this conference (a) A given protein may *appear in the serum with variable number of lipid components* attached to it from none at all to at least three, and perhaps four free and esterified cholesterol, phospholipids and possibly neutral fat (b) If number of lipids and interrelationship among them is constant then the quantitative relationship between the protein and lipid portion of the complex may vary and in this variation cause changes in sedimentation characteristics of which density

and size of aggregate appear especially important Our evidence that there are lipoproteins in normal human serum with differing degrees of complexity as far as number of lipid components is concerned has led to the first concept and the evidence for wide *variation in density of complexes in which the organization of the* lipid component appears to be constant is the basis for the second These concepts give to the protein portion of lipoproteins a role of great flexibility even versatility as lipid carriers permitting variation in lipid load both as to kind of lipid components and size of load Possibly part of the load may be taken on in one organ and another part elsewhere likewise unloading may be in stages and at different parts of the body This sort of role is in keeping with the general theories concerning plasma proteins as carriers formulated by Bennhold(6) whose concepts encouraged us in undertaking these studies

I must make clear that my associates and I are aware of still other possible interpretations of these mathematical relationships One possibility is that below the top zone there is one lipoprotein for each lipid component and more than one for certain of them and that for two or more the sedimentation characteristics are identical and concentrations of each in normal serum maintain such exact relationships within the group as to make it appear that they are combined into a single complex This impresses us as a poorer explanation for our data than that developed above

In our mathematical studies of normal centrifugate cholesterol esters appeared to constitute an important part of the highly elaborated Beta$_1$ (correlated) lipoprotein Yet in severe hepatitis only a small concentration of esters is found and this in part is still closely associated with this free cholesterol phospholipid (Figure 5) According to a rigid concept of molecular structure one is forced to hypothecate a separate complex which carries the cholesterol ester which has the same sedimentation characteristics as the free cholesterol phospholipid complex According to this interpretation the Beta$_1$ lipoprotein would consist of two separate components which have the same characteristics when studied by electrophoresis and in the quantity ultracentrifuge We lean toward *a concept which permits variability of structure of lipid complexes* and suggest that the Beta$_1$ lipoprotein in the zone of low medium density of normal serum is the carrier for four lipid components three of which are incorporated into the molecule according to the exact proportions and neutral fat according to variable proportions

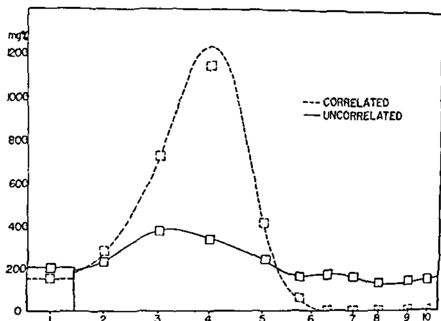


FIGURE 5 Distribution of two forms of phospholipid in centrifugate of one serum
lated phospholipid found by multi
1 87 and the uncorrelated by sub
iospholipid in each of ten samples
alculated by this method represents
40 per cent of total in contrast to the normal mean of 62 per cent

In hepatitis the ester component is diminished and the neutral fat is *greatly* increased This question cannot be settled until lipoproteins are separated from both normal and hepatitis serum in great purity and examined by multiple physical and chemical methods Some possibilities are given in the following

> Some of the possible explanations for peculiarities of what appears in normal serum as elaborately organized multiple-lipid complex Beta₁ (correlated) lipoprotein
>
> I Four different lipoproteins, one type for each lipid component found, with identical sedimentation characteristics, occurring in normal serum in such concentrations as to indicate stoichiometry for three components *Highly improbable*
>
> II Two different types of complex, each with at least two lipid components, one water soluble, the other insoluble, same sedimentation characteristics and proportional concentration in normal

A Free cholesterol — phospholipid complex

B Cholesterol ester neutral fat complex According to this possibility (a) would increase in hepatitis as (b) diminished Another modification of possibility II would have (a) carry three lipids, and (b) only one ester Both seem improbable

III Modification of I or II Two or more lipoproteins are joined by some loose physical bond which occurs either in the blood stream or during laboratory manipulation so as to give some characteristics of a single type of complex High concentrations of complexes and changes in suspending medium possible factors Mixing centrifugate with solution of sucrose or electrolyte in order to measure density of complex may be source of such error Worth serious consideration

IV A lipoprotein complex in which two forms of cholesterol and phospholipid are contained in stoichiometric proportions and neutral fat in variable proportions, a large proportion of the ester may be lost from the complex in hepatitis Improbable but meets the requirements of probability and our data somewhat better than others

The following is a classification of lipid complexes into three principal groups according to distribution in centrifugate of untreated serum

I The lipids of the cream layer

II The highly elaborated multiple lipid complex or group of complexes containing both forms of cholesterol and phospholipids in strict proportionality and neutral fat in variable proportions

III Lipids unrelated in distribution to free cholesterol (This is obviously a 'leftover' class) The two following sub groups have distinctively different curves of distribution

A Uncorrelated phospholipids Contains an average of 62% of the phospholipids in normal serum, and are greatly reduced in hepatitis *Extremely wide distribution in column, wide range in* particle size or density or both A "system" of complexes One definite component has a mean specific gravity of 1 053

B Uncorrelated cholesterol esters Two peaks in normal contain an average of 30% of esters in normal serum

Before I go into the question of deviations from the normal which we have observed in disease and in normal individuals under dietary stress it is necessary to mention variability in the normal curves of distribution for lipids which has been established by standard statistical methods The mean and standard deviation of concentrations in each of the 10 samples in our normal group is available(1) Sera from normal individuals under dietary stress and from patients with hepatitis and obstruction of the extra hepatic biliary tract show distribution curves which deviate so widely from the mean normal curves that there can be no doubt about significance After five days of complete caloric starvation there is a significant increase in concentration of lipids in the zone of low medium density and in hepatitis there is also an increase in the same zone but there are other peculiarities which we believe more important The peak of lipids in this zone is shifted upward in the tube where the specific gravity of the centrifugate has a lower value than in the normal In neither of these conditions is there any significant change in neutral fat concentration of the top zone (cream layer) In hepatitis sera there is a tendency toward deficit in all lipids in the lower one third of the tube Because hyperlipemia is common in hepatitis and it is desirable to view distribution of lipids independently of this increase in concentration we have compared normal and hepatitis serum when each value for centrifugate was expressed as per cent of concentration in original whole serum When our data are viewed in this fashion there is an excess of lipids in centrifugate of mean specific gravity of about 1 010 and a deficit in the bottom one third of the column Such a relative and localized excess falling between the cream layer and middle of the column was found even for cholesterol esters which in severe attacks may be found in diminished concentration in whole serum

In terms of our mathematically differentiated normal complexes there seems to be an excess of a lipoprotein in which there is an exact relationship between free cholesterol and phospholipids (Correlated phospholipids) In great contrast there is a deficit of the two heavier lipoproteins of simpler lipid composition one of which seems to have a special function as carrier of phospholipids and the other of esters in addition to an excess of low density lipids and there is an excess of neutral fat not in the cream layer

How can we interpret in physiological terms such changes? For excess concentration there are two general possibilities acceler

ated formation or slow removal or utilization and for deficit the converse Are deficit and excess possibly manifestations of a common disorder of mechanism? If abnormality is primary in one kind of complex which is it those showing increased or diminished concentration? One might guess that synthesis of the globulin essential for the simple heavy lipoproteins is a major fault in hepatitis and that in contrast the step from simple to elaborate lipid complexes is achieved by the hepatitis patient more readily than primary globulin synthesis Therefore the concentration of simple heavy units diminish as the more elaborate ones of lower density increase To carry the guess further there may be an economy in utilization of scarce globulin molecules in that each carries a bigger and perhaps more elaborate lipid load It would probably be best to say that in hepatitis there is either increased production or diminished utilization of low density lipoproteins and either diminished production or increased breakdown of high density complexes We guess that accumulation of low density complexes is related to slow utilization and deficit of high density ones to slow production

Our experience with labeling lipid phosphorus with P^{32} shows that in samples 2 to 6 there is often a proportionality between net gain and isotope and density of centrifugate (Figure 6) Such evidence suggests that there is an important relationship between physiologic activity and density of lipoprotein in this portion of the centrifugate

In the cream layer I failed to point out we find no difference in concentration of neutral fat between hepatitis and normal Where do these chylomicrons come from in the post absorptive state? It looks superficially as if the liver has very little to do with these chylomicrons If it does its function does not appear disturbed by our evidence

Best Dr Turner did you mean that the composition of your first layer contained in your centrifuge is the same as you would get in just an ordinary centrifuge?

Turner No It looks like the same material but as far as its chemical composition is concerned we have never compared them We do not know how much of the material in our top layer would come to the top with ordinary centrifugation

It contains chylomicrons I would guess that some of it would come up with ordinary centrifugation

plexes of similar lipid aspect may turn out to be our most satisfying contribution

Best This has been extremely interesting Dr Turner Those of us who have spent some years thinking about the physiologic or pathological significance of chemically separable entities now have to change our point of view and I take it that your interest is the physiological and the pathological significance of physically separable constituents

Turner Physically separable and chemically identifiable

Best Yes that s right too

Dr Hanger will you comment on this subject?

Hanger The interpretation of Dr Turner s interesting data depends largely upon one s concept of the serum complex I visualize it as a patterned equilibrium in which the innumerable constituents of serum bear selective relationships to one another forming compounds and complexes of varying stability This labile arrangement undergoes readjustments whenever one or more of the constituents are altered as in disease and also when the serum is subjected to various forms of physical or chemical stress Much of our knowledge of the nature of the serum complex depends upon the reproducible patterns of fractionation obtained when normal or abnormal sera are subjected under standard conditions to the effects of various solvents salt solutions electric fields and precipitation agents Dr Turner has utilized gravity to distort the relationships of the serologic components Complexes whose constituents are of similar gravimetric values and those with strong intrinsic attraction undergo presumably much less disruption than those having constituents with marked mass differences or with labile linkages It is most satisfactory that normal sera from different sources yield patterns that are reasonably comparable and these should prove useful as a standard with which centrifugates *from abnormal sera can be compared Dr Turner has already shown* significant changes in hepatitis serum One of the commendable features of his method is his using whole undiluted serum for study I should first like to ask him whether he thinks that with homogenization of his several fractions he could reconstitute the original serum I suspect that certain relationships have been irreversibly broken down and this would lead to permanent alterations in the structure of the complex

Turner We have done it and it goes back perfectly well

Hanger Perfectly well? Every physical property is the same?

Turner Of course not We have never studied every physical property of anything

It can be reconstituted very well as far as the Hanger and thymol turbidity tests are concerned

Hanger That is the point I think it is a very delicate dismemberment

Turner We are very proud of our gentleness

Hanger Yes I think you should be

This work gives rise to other points of theoretical interest First the effect of standing It is well known that serum preserved even at icebox temperature is not a stable entity Complement disappears The prothrombin complex is altered and certain enzymes of the serum continue to be active For example Sperry (8) has shown that on standing the free cholesterol of the serum tends to decrease and the esterified cholesterol fraction increases due to the presence of esterifying enzymes My colleague Dr Kenneth Turner has observed a diminution of this enzyme in parenchymal diseases of the liver and is studying its value as an index of hepatic dysfunction at the present time(9) He allows serum to stand 24 hours at 37° and if the free cholesterol has not diminished at least 30 per cent in that interval he assumes a deficiency of the esterifying enzyme which in turn indicates defective hepatic activity There may be other enzymes which also might affect the composition of the fractions Dr Turner obtains with centrifugation

Another phenomenon observed in preserved normal serum is the

fractions upon which a negative reaction depends These lipid rich stabilizing fractions lose these properties even more rapidly when separated from the serum complex I am curious to know whether any of Dr Turner's fractions rich in lipid proteins contain these stabilizing components

The absence of stabilizing components in the serum is also notable in hepatitis and other active degenerative diseases of the liver Clinical observations indicate that these labile lipid rich com

plexes are rapidly formed and rapidly destroyed in the body for a positive cephalin flocculation may develop within 24 hours after hepatic injury by intravenous typhoid injections in the human or 48 hours after experimental phosphorus poisoning in the monkey Confirmatory evidence for this rapid turnover of lipoproteins in the blood has recently been made by London(10) using tagged glycine this investigator estimates a half life of only one or two days for these substances in contrast to albumin and globulin with a half life of about 18 days Dr Turners method of fractionating serum may become even more significant when the distribution of certain labeled constituents of the serum can be studied

Best I was wondering—perhaps someone knows—does the Sperry enzyme which makes cholesterol esters hydrolyze them—under other conditions? I have forgotten whether that is true or not

Hanger Sperry did not study the hydrolyzing effect of the enzyme in human serum but cites the work of others to indicate that the reaction is a reversible one He showed the trend in normal serum on standing is continued esterification of the free cholesterol fraction

Best I remember Sperrys report(8) on that but I was trying to remember also whether he had changed the conditions and found that it then broke down the compounds

Hanger Sperry found that cholic acid derivatives depressed the enzyme and in dog serum (not human) found concentrations of cholic acid which reversed the reaction He also pointed out that the enzyme is inhibited by hemolyzed red cells

The point I was making is that the sera of patients with parenchymal liver damage show marked diminution of the esterifying enzyme

Best Any comments at this point Dr Turner?

Turner We made some observations on the effect of standing I am sorry I dont have those figures It was a couple of years ago that we did it My recollection is that the changes were not as rapid as you mentioned—30 per cent in thirty six hours

Hanger Dr K B Turner carries out his test at 37° for 24 hours

Turner At incubator temperatu[illegible]ch faster than at room temperature

Hanger Naturally

Turner We are quite certain that it does not appreciably modify our values here

Hanger It might in some of those 24 hour centrifugations

Turner We dont do them for twenty four hours

Hanger But you showed some on longer centrifugation than your standard one

Turner Sixteen hours is our longest one

Best What temperature is that?

Turner Twenty eight degrees The rotor that holds these tubes has about that temperature

Best Which gives a good opportunity for enzymatic changes

Gyorgy How do you take off the layers?

Turner We have this gadget which slices through the tube at successive layers beginning at the top and aspirate successive layers into a syringe

Best You have not done your homework Paul It is in the paper I read it last night on the train!

Gyorgy You are ahead of me

Turner This work is supported by the Commission on Liver Disease so Dr Gyorgy has listened to it repeatedly He is thinking about –

Watson Massive necrosis

Shorr If you add portion 11 to say portion 3 or 4 and then go through the same process do you get a pattern as if they were not influencing each other or is there any detectable influence?

Turner We have not done that

Shorr If you have a dynamic equilibrium that might be a way of seeing whether you could disturb the relationships between your different components

Turner We must have a similar density gradient to reproduce a given distribution and whatever fractions you take to mix and re spin must re establish the density gradient that we had the first time or else you won't repeat the first distribution

We have mixed different samples and determined the effect on the thymol turbidity test and the Hanger test The bottom layers are most active the top layer a poor second but the two mixed give the highest thymol turbidity values of any combination we used The mid zone material has little activity or may be suppressive to the thymol reaction

Stetten When I first heard of this work in Tulane it seemed to me that this technique which is confessedly extremely laborious and technically treacherous was doing for the lipids of the plasma essentially what the Tiselius pattern had done for the proteins of the plasma It was giving curves which were very difficult to interpret but which should prove useful in the further understanding of the mode of distribution of lipids in the plasma and alterations in these distributions under various circumstances

One of the most appealing features and one that has been mentioned — perhaps it should be stressed again — is the absence of the pretreatment of the serum I think this is often not considered and it should be borne in mind that many operations carried out on serum involve either initially the addition of salt or what is equally damaging the addition of water Perhaps this is not generally recognized but if you lower the ionic strength you certainly induce alterations in the protein constituents of plasma In this present procedure as I understand it the serum is taken as such and without any dilution without any addition of anything it is further studied

In regard to some of the discussion I should like to interject again as I have done on occasions in the past a note of caution about the use of the word equilibrium Dr Hanger has told us that plasma was in equilibrium and then he told us that if it stood it underwent changes I think these two statements are contradictory in the usual sense of the meaning of equilibrium" A system in equilibrium does not undergo spontaneous changes

Hanger Equilibrium of the moment

Stetten Equilibrium when it is finally achieved lasts forever This is a semantic point such as our friend Dr Fremont Smith was talking about

Hanger I dont think I was obscure

Stetten I would visualize an application of this technique to the study of artificially prepared variants I would be very much

interested to learn what effect the addition of a small amount of bile salts has on the distribution pattern

Turner We have not done it

Stetten Or other variables such as serum allowed to stand in contact for a few hours or dispersed with solid sterol ester or sterol – whether the sterol under these circumstances enters the cream layer or will it enter this medium density region simply on contact?

It would seem to me that by such experimental procedures one might gain insight into the origin of some of the fractions that are being studied

Turner We have done neither of the things that Dr Stetten has mentioned I think with an increase in our facilities we may be able to do them They certainly interest us a great deal

Dr Stetten was with us last winter and gave us a number of very valuable suggestions

Knisely At one point you mentioned separation and reconstitution of physical properties For a cross reference in the literature at the January 1951 meeting of the Macy Conference on Blood Coagulation and Allied Problems Dr Edsall and Dr Waugh agreed to put together a bibliography of the known kinds of forces which hold particles together They agreed to cover our total knowledge of such forces – London forces van der Waal forces valences and so on They will provide an entrance to that whole chemical bibliography and put it where we can get hold of it

So far the changes which you have described have been ascribed to liver I would suspect that you have not had a chance to do it but it might be fascinating to draw samples of blood from the arterial side of an organ and from the venous side and see what the differences might be and perhaps this should be done for different organs because it might not all be related to liver function

Turner I should like to ask you Dr Knisely how profitable you think it would be to use arterial and venous blood say from a femoral artery and vein with and without muscular exercise in that extremity?

Knisely Well speaking as a histologist now a hind leg consists of bone striated muscle collagenous connective tissue and the various structures of skin and a few more so you are going to get some type of combination of those

Turner This was not as an alternative to studying organs

Knisely Part of the whole story

Turner Yes

Is it likely that muscles utilize some of these lipid components at a sufficient rate under exercise to make the difference?

Knisely Isn t it wonderful that we don t know? There is so much to find out

Turner I am trying to get some emphasis here

Dr Watson suggested at one of the meetings of the Commission on Liver Disease Armed Forces Epidemiological Board that we study hepatic venous blood to compare it with either arterial or venous arm blood We are trying to find out in studying these stresses what would be the most useful situation under which to do that study We have some analyses of whole serum in which the hepatic venous blood is compared with arm blood We find little difference in the whole serum so we should like to get a situation that would be most profitable and most likely to show differences

Knisely We have all the techniques now of Cournand(11) for drawing blood samples from different specific parts of the body in healthy and in diseased men

Turner That is what we were talking about It is the one we used in getting the specimens just mentioned

Watson You might be interested in the method which Dr Holmes in Denver has been using It is a type of application of a London cannula to the portal and hepatic veins of the dog so that he can keep these dogs under I should say almost normal conditions over long periods of time He can sample their hepatic venous blood and their portal blood simultaneously and serially for long intervals These are ambulatory dogs in apparently normal condition

Turner That brings up the whole question of working on experimental animals which has such obvious advantages to working on the human as we have been doing The situation can be controlled so much better

We have been inhibited for two or three reasons

The first is that the volumes of serum required in our present procedure are so great as to make it necessary to use pooled serum

from more than one animal Secondly we would have to establish our values for normal patterns for the individual animal which is a considerable undertaking

There are tremendous advantages in going to experimental animals

Best I suppose it would be quite hopeless at this stage but I wonder what are the difficulties you get into supposing you take whole blood in a silicon tube rather than just serum Is it possible to do that?

Turner Yes I think so The cells would presumably occupy the bottom of the tube in a pretty solid mass As far as I know they would stay intact So the plasma would be stratified above We would use up a good deal of the column for the red cells I would say and the length of the column is fairly short already The cell mass might not change from a vertical to a horizontal layer when the rotor stopped

Best You do get quite a lot of changes during the clotting procedure which it might be interesting to avoid

Artom Dr Turner as a first approximation would it be possible to group together some of these fractions in order to have a simplification of the technique —

Turner Yes we have done something with that

Artom — and also in order to have more material for the various analyses?

Turner By cutting the tube where the centrifugate has the specific gravity of normal serum we can show by examining the two fractions separately most of the peculiarities between hepatitis and normal centrifugates

We are afraid we will miss something important and we stick to this very laborious procedure I think too much I think we ought to try simpler sampling more extensively

Stetten Under this high centrifugal force if there were red cells packed at the bottom would you not expel intracellular materials into the aqueous phase?

Best You might

Stetten I dont know

Best The cells remain intact under your forces?

Turner We haven't done it, but it seems to me that it has been done, and intracellular juice is squeezed out

Stetten Which would dilute the plasma

Turner The intracellular serum is squeezed out in that portion of the column

Artom It would be just like working with a dilute serum because water is expressed out by such a tremendous centrifugal force

Knisely Does free hemoglobin show up in your samples?

Turner We do know something about that We have seen in one or two instances where we failed to re centrifuge and get rid of all of our red cells, a little pocket, or plaque of red cells, or possibly hemoglobin, on the peripheral side of the tube at the bottom

I believe the characteristics of hemoglobin would make it collect there, as would red cells We have not tried to differentiate between red cells and hemoglobin

Watson Dr Turner, I was not clear whether you had studied the simple effect of sterile incubation, along the line of Dr Hanger's comments, let's say, for twenty four hours

Turner No, we have studied the effect of esterification of cholesterol in the whole serum standing at room temperature and 37 degrees, but the effect of incubation upon these ultracentrifuge patterns we have not studied*.

Silliphant Dr Turner [illegible] these sp[illegible] taken a specified number of ho[illegible] or [illegible]dard period of fasting required? S[illegible] ating, [illegible]bution patterns of the lipids [illegible] take the [illegible] say, a half hour or six h[illegible] after the [illegible] same?

Turner The standard time for drawing the blood is 12 or 14 hours after eating and we usually insist on the subject — most of these normal subjects are medical students — not taking obviously fatty food at the evening meal before

As far as the immediate effect of taking a lipid rich meal we have some observations on that which I did not mention taking a specimen of blood and then having the subject eat a meal rich in the ordinary fat and other lipids — no pure fats — and then taking samples at two four and six hours We have studied several subjects by some modification of that technique

The effects are chiefly in the cream layer The chylomicrons are greatly increased as is known and there may be some effects elsewhere in some of these patterns But the last time we studied those they did not seem to be very significant It may be that now that our standard normal distribution curves are better established we may be able to say that there are some changes elsewhere but they are very unimpressive by comparison with say the effects of five days of starvation

Knisely Is your instrument a commercial instrument and if so what brand is it?

Turner It is an air driven Beams type instrument The angle of the tube is 10 degrees from the vertical It is not available commercially in its complete form

Hoffbauer Dr Turner would you comment on the effect of quick freezing of the serum for storage and subsequent reconstitution?

Turner We first used quick freezing of the centrifugate in the tube and then cutting the tube and the frozen column in the lathe into segments and thawing out the individual samples We abandoned that for two reasons There is obviously some turbulence induced by thermal convection currents particularly in the upper part of the tube The cream layer is no longer a thin layer at the top it is pretty well mixed with some of the adjacent centrifugate Then there were turbidities that developed which suggested to us that stabilization had been interfered with We did not run down what those turbidities meant The samples in some instances never were fully clear so we abandoned that technique and we now sample without freezing

Hoffbauer That refers to quick freezing after you have centrifuged the specimen?

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Silliphant Dr Turner, were these specimens always taken a specified number of hours after eating or was a standard period of fasting required? Shortly after eating are the distribution patterns of the lipids changed? If you take the specimens say, a half hour or six hours after eating would the patterns be the same?

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* [illegible] divided into [illegible] r ap- [illegible] r the [illegible] com [illegible] nt in [illegible] line [illegible] ls as [illegible]

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Hoffbauer That refers to quick freezing after you have centrifuged the specimen?

Turner Yes

Hoffbauer My question related to quick freezing for storage before centrifugation

Turner Because of these turbidities from freezing when we do store it, which we do overnight regularly, we do it at 4 degrees Centigrade, and we don't utilize freezing at all We think 4 degrees Centigrade is better than quick freezing We have kept some a little longer than that, five days in one instance We could not see any difference It was not a controlled observation — that five days of storage

Best Dr Hoffbauer probably had in mind that it might be very convenient to store them for a while and then ship them as samples but you really have not settled that point

Turner No

Watson There is no doubt that freezing will affect the ordinary tests such as Dr Hanger was talking about It will change quite a few of them They are not reliable after a period of freezing

Hanger There is just the possibility of it The proof would be in the actual testing Freezing, for example, does not affect the cholesterol esterifying enzyme I think that Dr Turner would be the last to say that this is an exact measurement It is a pattern just like an electrocardiogram You may feel that you are dealing more with entities than I do I think you may have some entities there but you have used gravity to bring about altered relation ships and have torn them so that they take these contours

Turner Those are not my words sir those are yours We don't speak of tearing in this way We may do it We speak of our gentleness

And as far as entities are concerned I think the entities described by those correlation graphs are very genuine things What they mean how important they are physiologically how tight the bonds are that hold them in the shape that they have, I don't know, but they are very genuine affairs and have very exacting mathematical characteristics which are not accidental.

Hanger Well, I can conceive of a heavy protein being attached to a very light lipid subjected to your gentleness, and have them torn completely asunder, whereas a light lipid and a light protein would cling together under your field of stress That is all I mean

Turner As I understand it, the forces applied to individual molecules by the ultracentrifuge which determine molecular distribution are quite small as compared to forces necessary to rupture bonds within molecules

Best When you think of shaking serums with ether and alcohol, you must do a lot of tearing things apart

Artom Of course

Hanger That's the way I view it I think this is the most gentle of the manipulations

Best I do too

Hanger I think it is beautiful I am not criticizing it in any way, but that is really what you are doing You are separating the heavy constituents from the light Whether they stick together or not depends upon the firmness with which they are bound

Knisely For fun it might be worth while to put in the record that the blood in the body is continually flowing in concentric laminae, and there are assuredly stresses and tensions from lamina to lamina There is certainly a degree of adhesion – let's use a nonspecific word – between plasma and endothelium and plasma and each type of cell And those shearing forces operate all the time and probably would act – well, this is guessing – to pull some of the attracting forces apart, and certainly operate to maintain constant stirrings And these forces are determinants of the ease with which such things as bacteria come in contact with, say, white blood cells

It might be fun to think about that

Turner Do you think there may be some stratification of some of these aggregates, that some of these laminae become richer in those of certain physical characteristics than others?

Knisely I would certainly look for it in the formation of large phlebothrombi in humans who lie still for long periods of time

We are now looking for the substances that cement masses together thus making up thrombi It might be worth while to look there

Best It would be a little difficult to imagine how you are going to get your samples I have in mind the formation of methaemalbumin in hemolysis, where the hematin combines with the albumin

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Hanger Thats the way I view it I think this is the most gentle of the manipulations

Best I do too

Hanger I think it is beautiful I am not criticizing it in any way but that is really what you are doing You are separating the heavy constituents from the light Whether they stick together or not depends upon the firmness with which they are bound

Knisely For fun it might be worth while to put in the record that the blood in the body is continually flowing in concentric laminae and there are assuredly stresses and tensions from lamina to lamina There is certainly a degree of adhesion — let's use a nonspecific word — between plasma and endothelium and plasma and each type of cell And those shearing forces operate all the time and probably would act — well this is guessing — to pull some of the attracting forces apart and certainly operate to maintain constant stirrings And these forces are determinants of the ease with which such things as bacteria come in contact with say, white blood cells

It might be fun to think about that

Turner Do you think there may be some stratification of some of these aggregates that some of these laminae become richer in those of certain physical characteristics than others?

Knisely I would certainly look for it in the formation of large phlebothrombi in humans who lie still for long periods of time

We are now looking for the substances that cement masses together thus making up thrombi It might be worth while to look there

Best It would be a little difficult to imagine how you are going to get your samples I have in mind the formation of methaemalbumin in hemolysis where the hematin combines with the albumin

No amount of centrifuging, so far as I know, will separate them although I believe in other respects the combination is a very light one

Hanger What are the specific gravities of your two constituents?

Maegraith Well, albumin is what? Sixty-something, isn't it? And the other one is hematin It is quite a considerable thing

Watson Of course, that is a strict chemical bond

Maegraith There is some argument about that – whether it is chemical or physical Weise(12) believes it is a physical one, Fairley(13) believes it is a chemical one

The second thing I should like to know is does Dr Turner equate most of these effects to change in the function of the liver cell? Did I interpret that rightly?

Turner No I never went that far

Maegraith Well, it was my word "interpret" for myself

Turner I should like your comments

Maegraith It struck me that there was some substance in what Dr Knisely said I am thinking particularly of hemolysis, because my major interest is blackwater fever In hemolysis of that degree, and to some extent in severe falciparum malaria, there is quite an upset – I think I am right, Dr Hoffbauer – there is quite an upset in the cholesterol in the blood and the relations between the free and the ester cholesterol I wonder how much of that is due to the actual destruction of the hemoglobin and the breaking up of the red cells

There is no doubt that in malaria, in particular, there is a great deal of phagocytosis of red cells, both parasitized and unparasitized As you know, their surface charge is greatly reduced It is rather like bacterial immunity On about the eighth day of the disease there is a tremendous absorption of red cells by the phagocytes and consequent destruction and I believe possibly that may have something to do with the changes that one gets in the blood

I often wonder, as a physician, how far one should interpret these things as being related to liver, or not I think not only the liver function has to be considered but also the carriage of the materials to the liver cell – in other words, the liver circulation – I hope we can get on to that point later

Smetana With regard to Dr Turners work on serum lipids I want to say that we have been interested in histologically demonstrable fat in the liver in hepatitis We have found that in cases of hepatitis there is practically no fat in liver cells but a great deal of fat may be present in Kupffer cells Under normal conditions there is practically always some fat in liver cells but none in Kupffer cells

Hanger You mean extractable fat or demonstrable fat?

Smetana Histologically demonstrable fat We have not investigated the kind of fat which is present but we have observed this phenomenon rather consistently

Hartroft At autopsy or biopsy?

Smetana In biopsies

Turner What fat stains show that up best?

Smetana Routine fat stains such as Oil red O Sudan III Nile Blue Sulphate or Osmic acid

Shorr Have you studied any type of infection other than hepatitis? Does infection per se *involve* these fractions and if you will forgive the expression does the adrenal cortex have anything to do with it? We do know that certain of the plasma proteins are dependent upon adrenocortical activity

Turner For some reason—I forget why it was—we studied fairly severe tuberculosis two or three cases of it These did not resemble hepatitis We have not studied the very acute infections like lobar pneumonia

As far as the adrenal cortex is concerned we have studied 2 patients with rheumatoid arthritis first before they had cortisone and then afterwards They were cases that had increasing lipid concentrations under the conditions in which we studied them—perhaps as a result of therapy of course As I remember this low medium density complex showed perhaps the greatest effect This showed little resemblance to hepatitis

We have not studied a case of Addisons disease I wish we could I hope we can yet

Artom Did you study any case of lipid nephrosis?

Turner Yes we have studied such cases There is hyperlipemia in hepatitis but the distributions are quite different

Artom That is a most interesting condition, I believe for the udy of the relationships between lipids and proteins in plasma

Shorr And you refrained from using ACTH in your patients ith hepatitis?

Turner Yes I should also like to mention that in conditions like phrosis we have such extreme hypoalbuminemia that we add mmercial albumin before centrifugation to bring up the total otein concentration

It happens in these cases of acute hepatitis the total protein con ntration is pretty near normal The albumin globulin ratio is ually abnormal The density gradient is sometimes different— finitely different in hepatitis serum and normal But we have not en able to attribute any of these differences in pattern to the fferences in density gradient

Shorr Do you not find a hypovolemia in most of these cases?

Turner No we made no measurements of volume We should e to do that

Popper Have you by any chance determined the concentrations fat soluble vitamins especially of vitamin A in the different yers? The liver is the main store for vitamin A and influences the asma vitamin A level Therefore the behavior of vitamin A in e different levels as related to the other lipids may be of interest so in view of what Dr Smetana mentioned

Turner No we made no measurements of anything like vitamin Neither did we see a difference We should like to do that

Popper The vitamin A concentration can now be determined very small samples(14) The findings so obtained could be cor lated with the histologic distribution of vitamin A as studied by orescence microscopy(15) This could possibly aid in the explan on of the disturbances of the vitamin A metabolism and in alogy with similar observations on lipids, of the disturbance of e fat metabolism in liver disease In support of what Dr Smetana s just said about fat distribution there is a characteristic change the histologic distribution of vitamin A in hepatitis and in infec ons in general The Kupffer cells appear loaded with vitamin A contrast to the small amounts of vitamin A fluorescence in the er cells

Best You would really like to have your protein pattern identical all your runs and then study the lipid changes

Turner Yes

A very simple experiment that we want to do but just have not done is to take a typical hepatitis serum and dilute that with albumin solution so as to restore the gamma globulin and albumin both to about the normal value I think we could do that pretty accurately It will be interesting to see what we get That is on our list and has been on for some time both other things have crowded it out

Shorr Is it possible that there might be an appropriate enzyme inhibitor which would be effective at very low concentrations This might eliminate the objection that was raised to changes in the course of your experimentation from handling Perhaps Dr Hanger would know of work on enzyme inhibitors with respect to that phenomenon

Hanger I don't know which one I would suggest I think Dr Turner is to be congratulated on not trying any artificial systems, just adding insoluble lipid suspensions to a serum can change its physical properties That is in itself a distortion of what I still like to call an equilibrium Dr Stetten

Best Dynamic equilibrium

Hanger Dynamic equilibrium Yes if you assume the enzymes in the serum then I think it is a dynamic equilibrium

Best Other questions?

Dauphinee May I ask Dr Turner if he has made any observations on heparinized plasma and what happens to the fibrinogen?

Turner No Dr J P Peters was in New Orleans this winter and was kind enough to go over these data with us and he was very much interested in heparin effect We have left it alone

Hartroft Does the turbidity which develops after freezing drying alter the pattern of equalization? Have you done duplicate determinations on a single sample before and after freezing-drying? Have you any data concerning the effect of freezing?

Turner No I do not We made no effort to study that We just accepted the turbidities that developed there as a warning that we should avoid freezing Now whether the warning was justified I don't know

Hartroft Perhaps it might be helpful to learn whether or not the turbidity associated with freezing affects the patterns you obtain This might permit standardization of some of these results

Turner Yes

Hartroft For example, I could send you a few samples

Best He has quite a lot of samples

Turner I would be very much flattered if anyone would like to send me a sample But we do turn them out very slowly. There is no question about that

Best Any other questions? Dr Hanger, have you any further comments?

Hanger The antigenicity of native proteins may be affected by the lipid complexes of the tissues A number of years ago I demonstrated(16) that saline suspensions of 6 day chick embryo tissue injected into guinea pigs failed to sensitize the animals But an equal quantity of the same material after extraction with alcohol sensitized quite readily It would be interesting to know if proteins in Dr Turner's lipid rich fractions are as antigenic as those in the lipid free layer

Turner It opens up a subject that I intended to mention and would have done so if I had not mentioned so many other things and that is in an effort to get more specific identification of proteins we are definitely planning this coming year to try to establish immunological methods of separation and analysis of these lipoproteins We are going to begin with this low medium density one and try to purify it by differential centrifugation We have done enough to know that considerable purification can be carried out there Whether the proteins are still with it or not we don't know yet That is crucial

Dr Henry Kunkel has pı ... abstract ... g that he
has developed an antiserum ... ipoprote ... tigenicity
depends upon the protein ... the lip ... the mole
cule(17) He has not publı ... on tha ... I know

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Shorr With respect to the question that I think Dr Hanger has been raising have you ever combined an entirely normal sample with a hepatitis sample and then exposed them to the same procedure to see whether by any chance you get the results which would be anticipated if they were not interreacting?

Turner No we have not I think it would be fascinating to do that

Watson How did Kunkel get his antigen the beta lipoprotein that he used?

Turner By precipitation with zephiran chloride

Best Have you any general comments Dr Turner including this part of our discussion?

Turner May I bring up one additional item that I did not mention before?

We are much intrigued with the material in the zone of minimum concentration We suspect that that is a small molecular material which is not affected by centrifugation and it is rich in phospholipids and in what we call neutral fat

I think the possibility that it plays a very special role in getting through difficult membranes is stirring to the imagination But if Dr Fremont Smith had not made his speech this morning I don t think I would have mentioned this But it seems to me that is a good one to go through the artery walls If the artery walls are nourished by lipids as some people think that would be a good one to furnish the normal nutritive lipids to arteries and perhaps that keeps us from arteriosclerosis There is so much excitement about lipids causing arteriosclerosis I should like to suggest very lightly here that maybe there is a lipid component identifiable by this procedure that keeps us from having arteriosclerosis Maybe it also goes through the blood brain barrier and maybe it is a nutrient for the brain

Now I think I will quit after that

Best Dr Knisely has a short communication for the record

Knisely This note is essentially the material I wrote you

For some time we have been collecting asserted functions of the liver such as the storage of water and storage of glycogen

When I went to medical school there were about thirty such named functions Right now we have five pages of them mimeographed

Some time ago a Ph D in biochemistry working with me found in a library survey about five hundred chemical reactions asserted to occur in the liver They have not been classified yet

Also a separate group of medical students have been making a list of the liver function tests used in clinics And thus far they have 97 listed liver function tests They have 2600 bibliographic references covering a ten year period and in a first survey of these have found 97 listed liver function tests

And now for the purpose of asking the privilege of putting this material in the record The liver function tests do not necessarily test for known functions of the liver so the liver may well have many as yet unknown functions which are reflected in the tests And there are certainly large numbers of functions for which we have no tests

One purpose is to get these two lists out where many people can add to them correct them and think about them

The lists* follow

Asserted Functions of the Liver

I *Carbohydrate metabolism*
- a Storage of glycogen
- b Maintenance of normal blood sugar level
- c Gluconeogenesis – formation of glucose from substances other than carbohydrate
- d Conversion of other sugars into glucose or glycogen
- e Glyconeogenesis

II *Metabolism of nitrogen compounds*
- a Storage of protein
- b Some storage of amino acids
- c *Deamination of amino acids*
- d Urea formation
- e Uric acid destruction
- f Formation of proteins especially fibrinogen
- g Synthesizes some amino acids
- h Formation of antibody proteins?
- i Uric acid formation
- j Purine synthesis
- k Glutathione metabolism

* The lists were not presented to the participants at the time of the Conference —*Editor*

- l Transamination
- m Nucleic acids
- n Flavine metabolism
- o Creatine metabolism
- p Heparin formation
- q Miscellaneous

III *Metabolism of lipids*

- a Storage of fat phospholipid and cholesterol
- b Desaturation of fats
- c Formation of ketone bodies
- d Synthesis of fat phospholipid and cholesterol
- e Affects blood cholesterol level and ester cholesterol fraction
- f [illegible]
- g [illegible] acids
- h
- i

IV *Formation and secretion of bile*

- a Synthesizes bile salts and bile pigments
- b Varies rate of secretion in response to digestive demand
- c Excretes foreign substances in bile
- d Effect of bile acids on hepatic blood flow

V *Relation to blood formation*

- a Stores anti pernicious anemia substance — pernicious anemia type of blood picture may occur in certain liver diseases
- b Disintegrates red blood cells breaks down hemoglobin and conserves the liberated iron
- c Stores exogenous iron and copper
- d Forms blood proteins and other essential constituents of blood i e prothrombin anti thrombin and anti prothrombin
- e Forms red blood cells in foetal life
- f Anti heparin factor

VI *Relation to water balance of body*

- a Removes excess water from blood i e buffers against abrupt blood dilution
- b By storing or releasing small amounts of blood it continuously regulates circulating blood volume
- c In frogs it also regulates the peripheral red cell count
- d Removes water from blood at onset of fever — aids in blood concentration

VII *Metabolism of inorganic salts*

- a Liberates potassium on stimulation of sympathetics or after epinephrine
- b Has a part in calcium balance — decreased calcium storage is present after liver damage
- c Aids in regulating pH of blood — outflowing blood is more alkaline than inflowing
- d NaCl metabolism

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g Formation of glycogen from odd chain fatty acids
h Fat content increases during fat mobilization
i Oxidation of fatty acids

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c Aids in regulating pH of blood — outflowing blood is *more* alkaline than inflowing
d NaCl metabolism

- e P metabolism
- f Br metabolism
- g Zn metabolism

VIII *Detoxifies foreign organic compounds*

- a Various substances may be detoxified by oxidation reduction methylation acetylation or conjugation with glucuronic acid cysteine, glutamine or sulfate

IX *Vitamins participation in vitamin metabolism*

- a Vitamin A
- b Vitamin C
- c Nicotinic acid
- d Vitamin B6 (Pyridoxine)
- e Nicotinamide
- f Vitamin K
- g Inositol
- h Biotin
- i Pantothenic acid
- j *Folic acid*
- k Vitamin M

X *The Kupffer cells in the blood vessels of the liver remove particulate foreign matter from passing blood*

XI *Specific dynamic action of compounds in liver*

- a CHO
- b Protein
- c Fats

XII *Metabolism of sulfur compounds*

XIII *Hormones and the liver*

- a Detoxification by a hormone
- b Estrogens
- c Miscellaneous

XIV *Antibody formation in liver*

XV *Enzymes of liver*

XVI *Acid – base balance*

XVII *Lymph formation in liver*

XVIII *Miscellaneous*

Classification of Liver Function Tests

Compiled by Max Culp Roy Ellison David Hull Francis McCorkle William Miller and William Nelson 1949 50

1 Acetone test
2 Alcohol with reference to
3 Althausen test
4 Amino acid tests
5 Ammonia test
6 Atabrine test
7 Azorubin azotemia azouria tests
8 Bengal rose test
9 Bile acids and salts
10 Blood diastase
1[illegible] ent including – tion in blood

b tolerance tests
c excretion tests
12 Blood proteins
13 Bromsulphalein bromophthalein Rosenthal or Rosenthal White
14 Burger test
15 Carbon tetrachloride test
16 Cephalin *cholesterol* flocculation (Hanger test)
17 Cevitamic acid test
18 Cholecytography
19 Cholesterol
20 Chloral clearance
21 Cinchophen oxidation
22 Citric acid
23 Cobra venom as test
24 Colloidal gold test
25 Colloid injection
26 Congo red test
27 Cyanotic induration
28 Duodenal content and tests
29 Dye or stain tests (non specific)
30 Epinephrine
31 Estrone clearance test
32 Fats and lipase
33 Felix Tesla test
34 Fuchsin test
35 Galactose tolerance test or Donath Frisbacher test and related papers
36 Galdi test
37 Gelatin and water tolerance test combined
38 Glucose or *dextrose* test
39 Glucoronic acid
40 Glycocoll *test*
41 Gros reaction test
42 Hay's test *stalagmometry* and surface tension of urine
43 Hemoglobin —
a tolerance
b breakdown
44 Hippuric acid
45 Icterus
46 Indican indole
47 Indigo carmine
48 Insulin
49 Iodine
50 Lactic acid and salt tests
51 Levulose
52 Leukocytic test
53 Liver *function* in arsphenamine treatment
54 *Maillard's* coefficient
55 Methylene blue
56 Millon reaction test
57 Mineral water test
58 Novasurol *test*
59 Oil test
60 Oxalemia and oxalic acid
61 Paul von Veigh test
62 Phenol sulfoephthlein
63 Phlorizin
64 Pletnev Sokolnikov test
65 Polysaccharide tests
66 Porphyrinuria
67 Procaine hydrochloride
68 Prostigmine and acetylcholine
69 Pyloric closure reflex
70 Quinine
71 Roch test
72 Rosenthal tests (non specific)
73 Santonin test
74 Sodium salicylate test or salicylate test
75 Sodium chloride
76 Sodium nitrate
77 Sucrose test
78 Sulfanilamide *with reference* to
79 Sulphoconjugation
80 Takata Ara reaction
81 Takata reaction
82 Takata Jezler
83 Tetra phenalphthalein halide tests or Rosenthal tetraphenolphthalein halide *test*
84 Thymol turbity
85 Tribrom *ethanol* narcosis
86 Trypanocidal titer
87 Ucko test (modified Takata Ara)
88 Unconjugated ketocholanic acid
89 Urea test
90 Uric acid test
91 Victoria blue test
92 Vitamins
93 Volhard water test
94 Weichbrodt's Reagent
95 Weltmann reaction
96 Wine test
97 Yakriton

NOTE There are numerous *references* that are unclassifiable as to specific liver function tests and others that contain several tests in one reference

I suspect that one real good project now would be to have good biochemists begin to comb the literature and see how many known chemical functions of the liver exist, then classify them in terms of chemistry and see how much real knowledge actually exists This follows Dr Fremont Smith s statement that one of our pur poses here is to get together ideas that are widely scattered in different named fields of science

Best Any other general comments?

Maegraith May I make a comment about the function of the liver cell?

Best Yes indeed

Maegraith It seems to me that there are two ways in which the activity of the liver cell may be affected One is by a change within the cell itself and the other is as I said before by a change in the environment of the cell especially in its blood supply

The failure to appreciate this may account for some of the con fusion physicians feel when presented with a series of liver function tests for interpretation One test which is often difficult to interpret is the bromsulphalein retention test which does not by any means always parallel other tests In fact it doesn t often parallel anything I think this is because it reflects something that is going on in the liver which the other tests do not We are working in Liverpool at the moment on heart failure cases in which the liver enlarges and later recedes with treatment

As I see it retention of bromsulphalein in the plasma can occur if the blood containing the dye is not getting to the active cellular area owing to some change in the intrahepatic circulation with the result that the liver cells are unable to remove it Under these circumstances retention of the dye may occur when there is little or nothing wrong with the actual function of the cell I think we may have to learn to separate true failure in liver cell function from effects produced by changes in the intrahepatic blood flow

It was on the basis of this sort of argument that we started our work on the circulation of blood in the liver in an attempt to find out whether it is possible for the blood flow through the organ to change in such a way that the total surface of functioning liver cells exposed (directly or indirectly) to a particular substance in plasma can be varied considerably

Knisely May I state a point of strict agreement? Just a superficial survey of the liver function tests, without any attempt to learn too much about any one of them, begins to show one thing like this

There are some tests in which something is put into the blood stream, and then rates of disappearance of that substance from the blood stream are measured And in every such case as that, the number of molecules of the injected substance which can be removed from the blood per hour, or per day, or per period of time, directly depends (mathematically) upon the *concentration* of the molecules in the blood passing through the liver, and the linear rates of flow through the vessels And the number of molecules removed depends upon the degree of stickiness of that molecule to the lining of the liver sinusoids and whether the molecule is retained after it bumps the wall

So there are three classes of variables one, the concentration, two, the rates of blood flow, and three, the rates of attachment

At present our curves show the sum of three known variables in operation and usually they are not treated separately I suspect that it might be possible to select two or three of these liver function test substances and by doing (I guess I do not know quite enough mathematics) – by doing some combinations of "simultaneous equations" to get the rates of blood flow through liver from two simultaneous tests and then knowing the concentration of the test substances in the blood it should be possible to interpret the tests in such a way as to show the degree of adhesiveness of the molecules to the inner surfaces of the liver to which they are attached

Best You say there are three points One is the concentration of the substances in the blood, and one is the rate of blood flow through the liver

Knisely Yes

Best And one is the extent of adhesiveness

Knisely Yes, by which is meant the ability of the liver cell surface to become attached to and hold the ion or molecule

Best To pull it out.

Knisely Yes

You see, we are dealing with a three variable problem in mathematics but so far we have treated it as a one-variable problem We

are able to measure one variable, the concentration of substances in blood and by simultaneous equations we may be able to separate one variable off and measure it, namely the rate of blood flow And then we should be able to calculate the other variable, that is, the rates of adhesiveness Does that make sense?

Maegraith There is a very interesting piece of work going on in San Francisco by Dr Hardin Jones in Lawrence's department, in which chromium phosphate tagged with P^{32} is injected intra venously and the rate of blood flow through the liver in the intact animal is determined by the disappearance of the tagged phosphate from the peripheral circulation Knowing the blood volume, keeping a check on that so that it is constant, you can work out by a simple dy $\times$ dx the disappearance of this substance from the peripheral blood

It has been shown that about 95 per cent of the phosphate is taken up by the Kupffer cells, so that the rate of its disappearance from the peripheral blood gives an approximate record of the flow through the liver

Best That is, in going through the liver, chromium is taken out?

Maegraith Well, it is taken out progressively on a sort of epsilon curve, and it can be calculated back mathematically quite easily It is simple enough for me to do it

We have put up an Aunt Sally We have said to ourselves, "This bromsulphalein story is essentially tied up with the circulation and not with the liver cell function" We intend to compare the rate of disappearance of this radio phosphate from the blood with the rate of disappearance of the bromsulphalein under controlled conditions, both in the intact animal and in the perfused liver where we can control the actual blood flow through the organ

Watson There is one factor which bothers me about this whole question of substances presented to the liver cell in relation to the circulation I think it is made more complex by the fact that bromsulphalein, for example, is returned by the lymph through the thoracic duct lymph into the blood How much of that bromsulphalein has actually been in the bile, I don't know, but it seems quite likely that some of it has actually gotten into the bile and has been regurgitated back across the cholangiolar epithelium and thence back into the blood

I feel quite certain that this is also true for many other substances that we think of with relation to hepatic excretory function, for

example bilirubin sterocobilin We have observed in dogs with thoracic duct fistula that if stercobilin is given intravenously just like bromsulphalein some of it immediately comes out in the lymph that is in a dog that has the common duct tied or has liver injury from carbon tetrachloride

Maegraith I think the important part is really just to make this point I was told at the beginning that it is a good thing to stick my neck out and I am doing it I think it is a very good point to make that we should think of something other than just the func tion of the cell and one of the obvious things is the circulation *And I think it is a good general principle to remember too that* the circulation in an organ is not just a bunch of tubes which are taking blood to and from that organ but an integral part of the organ just as much as the epithelium is Not only does the circula tion of one organ differ dynamically from another but I believe the physiological properties of the vascular endothelium are also different

Shorr Wouldn t you have to consider also the oxygen saturation of whatever flow there is?

Maegraith I am afraid I am talking too much

Knisely May I say one or two more words here which are definitely related?

Members of our laboratory currently believe that the liver sinu soid lining is a complete cylindrical lining and that there is a perisinusoidal space outside of it which space connects to and drains into the lymphatic circulation There is a published diagram of this (18a)

on

an

start thinking about the substances which are accumulated by the sinusoid lining von Kupffer cell as separate from those substances which are freely diffusing through the sinusoid lining

The rate of lateral diffusion of ions and small molecules through the sinusoid lining may be very rapid as compared to the longi tudinal movement of tissue fluid or lymph in the perisinusoidal space

Now nondigestible materials which are accumulated in von Kupffer cells when stored in a sufficient quantity begin to blockade

the acceptance of any other substances by the von Kupffer cells This is the so called physical blockade

I don't know enough about hepatic parenchyma cells to know whether they can be blockaded, whether for instance, one can give them enough glucose so that they won't take any more

Artom There have been several attempts to blockade the liver cells with a variety of substances in suspension or in colloidal solution but I do not think that there is any evidence for a functional blockage as the result of the anatomical blockade

Knisely Are you talking about the von Kupffer cells?

Artom Yes

Knisely Yes, that is, no one has shown a full blown functional blockade of the von Kupffer cells This word "blockade has several meanings Some persons talk about filling the von Kupffer cell so full that it cannot take any more — the physical blockade Others talk about filling it up so that it cannot make any more specific immune proteins, which is a different idea

Let me say one other thing There appear to be three permeability phases of the sinusoid lining This is published The following is taken, without contained references, from Knisely, Bloch and Warner(18b)

The tubular sinusoid lining membrane, which is contractile throughout its length, and each cell of which is a phagocytic von Kupffer cell has three distinct permeability phases In one, the individual red cells enter the sinusoid separated by small volumes of plasma, and the distance between the red cells does not become visibly less as the column of cells passes along the sinusoid During this phase not much if any fluid is passing out through the sinusoid wall into the perisinusoidal space and thence to lymphatics The liver is probably forming but little if any lymph(19) When outlet sphincters close at such a time, the sinusoids of these outlet sphincters store whole blood

During another extremely permeable phase the sinusoid lining membrane is probably permeable to all the colloids of the blood As the red cells pass along the sinusoids they come closer and closer together until the central two thirds, half or third of the sinusoid contains only packed red cells moving sometimes slowly, sometimes rapidly. If each outlet sphincter remains partly closed the sinusoid systems remain in a "continuous filtration phase" such

as sometimes occurs in spleen sinusoids and almost all the blood plasma is continuously separated from the blood cells. If outlet sphincters close at such a time the sinusoids of these outlet sphincters store highly concentrated blood cells As the hydrostatic [illegible]

to nearly zero volume against these proteins own inwardly directed osmotic attraction it is necessary to conclude that when the red cells are packing tightly together the blood proteins are all or nearly all passing out through the sinusoid wall During this phase almost all the plasma of the blood passes into the perisinusoidal spaces thence to lymphatics The sinusoid linings sometimes remain in this most permeable phase when the flow into them is very fast which is evidence that this permeability phase is not simply a result of stagnant anoxia

When frog hepatic sinusoid linings are in this most permeable phase the rate of formation of lymph must be proportional to the rate of blood flow into the sinusoids This agrees with the finding that dog and cat livers can and frequently do form large amounts of lymph which contains about as much protein as blood

The frog hepatic sinusoids also exhibit a third "[illegible] permeability phase" during which the linings probably are permeable to proteins of smaller molecular weight such as [illegible] but probably are not permeable to the larger ones such as [illegible] globulins or fibrinogen When the linings are in this [illegible] moving red cells come somewhat closer together but do not [illegible] tightly together even when the outflow is artificially [illegible] and the pressure in the sinusoids is artificially raised [illegible] normal level Bloch 1940 found that when certain [illegible] of acetyl beta methyl choline chloride were applied to [illegible] of frog liver the sinusoid linings went into this [illegible] permeability phase Whenever outlet sphincters close [illegible] soids are in this phase the sinusoids of those closed outlet [illegible] store partly concentrated blood cells

We have traced literally hundreds of sinusoid [illegible] from [illegible]

the liver has not been injured by rough experiment [illegible] red cells are not found outside the sinusoid linings

The perisinusoidal spaces are frequently visible at 400 to 600X but are very narrow, even when fluid is passing rapidly out through the sinusoid walls When fluid is not passing into the perisinusoidal spaces the sinusoid walls lie against the hepatic cell cords obliterating the perisinusoidal spaces just as the spaces between the leaves of a book are obliterated becoming potential spaces when the book is closed

In a given microscopic field under uniform experimental conditions the sinusoid linings may change from any one of these three permeability phases to any other, or may stay in one of these phases for several hours or may go through rapid or slow cyclical changes from one phase to another By combinations of the permeability phases and shutting and opening of the outlet sphincters various controlled amounts of blood cells and of whole blood are stored in the liver and various controlled amounts are released The fact that all the permeability phases may alter while they are observed and that adjacent sinusoids may be in different permeability phases and each change to another phase and that they go through cyclical changes are evidences that no one of the permeability phases results from conditions imposed by the anesthetic the operation or the transillumination technique

There is one more overwhelmingly important point on the sinusoid lining membranes of the liver which we must now keep in mind Dr Hans H Ussing who is a student of the late Professor August Krogh and a member of the Zoophysiological Laboratory at the University of Copenhagen in Denmark has been doing a series of experiments in which he finds that some kinds of living membranes can accept some kinds of ions from one side of the membrane and then pump these ions out to the other side of *the membrane The membrane thus adds energy* to the system and can initiate and maintain a higher concentration of the ion on the side to which it is sending the ions than on the side from which the membrane is receiving them

Best The membranes have glandular function

Knisely Yes in common parlance persons in this field talk about secretions of ions

So that world of literature should be brought together so that we can use it in thinking about and testing the functions of the hepatic sinusoid lining membranes Two reviews have appeared(20,21)

Popper It is noteworthy that, in contrast to the original belief, bromsulphalein is not first taken up by the Kupffer cells and transmitted to the liver cells Recent observations by Mendeloff and his co workers(22,23) indicate that bromsulphalein (and also rose bengal) seem to go from the blood stream directly to the liver cells and can never be demonstrated in the Kupffer cells

Knisely What method is used to look for the dye in the von Kupffer cells? Does a low detectable concentration of dye in the von Kupffer cell necessarily indicate that the dye does not go through the von Kupffer cell? Might each molecule of dye pass through the cell so rapidly that at no time are there very many molecules in the cell?

Popper By fluorescent microscopy

Maegraith Of course, that does not really matter That does not affect the argument, because the point is that you get less bromsulphalein reaching the liver cells (whether through the Kupffer cells or not) because there is less blood circulating in the active area

Popper However, differences in the behavior of two substances could possibly indicate a difference in takeup by the Kupffer cells and in takeup by the liver cells As to the question of the blockade of the liver cells I would like to refer to observations of Williams (24 25) that the damaged liver cells (i e in carbon tetrachloride intoxication) take up larger amounts of dye than normal liver cells specifically rose bengal He was able to show that the damaged liver releases the dye much slower than the normal liver This delayed clearance of the liver should be considered in the problem of blockade

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SECTION II

MECHANISM OF ACTION OF LIPOTROPIC FACTORS IN ANIMALS*

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SINCE SEMANTICS WAS mentioned this morning may I start with a little discussion in semantics in an attempt to define for you what I am going to talk about

Etymologically the term lipotropic would apply to any factor modifying the metabolism or the distribution of fat in the body Conventionally it has been most generally used with the same meaning which was originally intended by Best and his co-workers(1) that is to designate specific dietary factors which prevent or cure the fatty infiltration of the liver Strictly speaking this definition would exclude certain nonspecific effects such as those of a low temperature in the environment Hormones and other nondietary factors should also be excluded Accordingly I *shall not discuss lipocaic which is possibly either* a hormone or a proteolytic enzyme Moreover any suggestion on its mechanism of action should wait until the chemical and physiological identity of this factor (or factors) has been determined

Inositol is included in our definition since it is present in many materials of both animal and vegetable origins which make up our *diets There seems to be general agreement* upon the lipotropic activity of inositol although such activity is apparently of a moderate degree and can be clearly demonstrated only under certain experimental conditions The *only* indirect suggestion *for* explaining its mechanism of action has come from the fact that inositol like *choline is* a component of phospholipids *and that the presence* of inositol containing phospholipids has been demonstrated in animals

* [illegible] k done partly with the aid of [illegible] contracts between [illegible] Gray School of [illegible] osium on Lipids [illegible] New York September 1950

in plants, and in micro organisms It should be pointed out, however, that unlike choline, inositol is apparently a minor constituent of the phospholipids of the liver

In addition to inositol, choline and compounds containing pre formed choline, the lipotropic factors commonly present in our diets are betaine, methionine, and proteins Other lipotropically active substances, such as beta-propiothetin and dimethylethanolamine, have been found occasionally in biological materials, but certainly are not widely distributed in nature With the exception of dimethylethanolamine all of these naturally occurring compounds contain biologically transferable methyls and thus may contribute to the synthesis of choline in the body The effects of the addition of folic acid and vitamin B_{12} to low choline diets are also probably due to a role of these vitamins in the synthesis and transfer of methyl groups Dimethylethanolamine does not contain "labile" methyl groups, but differs from choline having just one less methyl It may therefore be regarded both as an immediate precursor in the biological synthesis of choline and as a very close analogue of choline

Among the large number of synthetic products which have not been found in nature but exert a lipotropic action when they are fed to animals, dimethylthetin is an extremely effective methyl donor All other compounds, such as triethylcholine, arsenocholine, thiocholine, homocholine, etc, have a molecular structure similar to that of choline It seems, therefore, that if we knew the mechanism of the lipotropic action of choline, we could also easily account for the effects of most other lipotropic factors by assuming that they act as biological precursors of choline, or that they favor the synthesis of choline in the body, or that they may substitute for choline in the chemical reactions resulting in its lipotropic effect Accordingly, my discussion will be chiefly confined to the mechanism of the lipotropic action of choline

EARLIER VIEWS ON THE MECHANISM OF THE LIPOTROPIC ACTION OF CHOLINE

At this point let me say that I was delighted when I received from Dr Best the invitation to prepare this presentation However, I could not help thinking that if this invitation had come ten years earlier my task would have seemed much easier Indeed, in the years between 1932 and 1940, during which the lipotropic effect of choline and of its precursors was described and studied ex-

tensively a number of other findings had also been reported which appeared to substantiate the earlier view that phospholipids (or at least certain types of phospholipids) were intermediary compounds in the absorption, transport, and metabolism of fatty acids

Among these results I shall mention here only those obtained in our earlier studies with the aid of radioactive phosphorus as a tracer(2) In these experiments, the incorporation of radioactive phosphorus into the phospholipids of the liver and small intestine possibly also of the kidney, was higher in rats fed a diet rich in fat than in rats on a fat free diet On the other hand, the presence or absence of fat in the diet did not affect appreciably the rate of formation of phospholipids in all other tissues

We thought that these findings which are summarized in Figure 1 could be interpreted by the hypothetical distinction of two

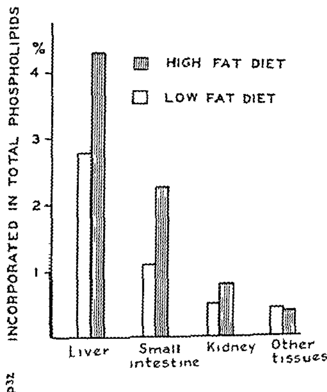


FIGURE 1 The incorporation of isotopic phosphate into the tissue phospholipids of rats fed respectively high and low fat diets The rats were maintained for 4 days on purified diets containing 25 per cent casein Reprinted by permission from Artom C Sarzana G and Segrè E *Arch internat physiol 47, 245 (1938)*

classes of phospholipids with different functions Tissues such as the liver the intestinal mucosa probably also the kidney and blood plasma would contain a high proportion of "lipometabolic phospholipids" specifically involved in fat metabolism whereas most of the phospholipids present in other tissues would represent chiefly essential constituents of the cell ("cytoplasmic phospholipids") Since choline is lipotropic but ethanolamine apparently is not the lipometabolic phospholipids most likely should be lecithins In this respect Welch(3) had already pointed out that the arsenic analogue of choline is incorporated into the phospholipids of the tissue and is lipotropic but does not contain biologically labile methyls thus indicating that the intact molecule of choline is involved in the lipotropic effect Furthermore in 1939 the findings of Perlman and Chaikoff(4) that single large doses of choline betaine or methionine markedly stimulated the incorporation of isotopic phosphate into the liver phospholipids seemed to give strong support to the idea that choline is lipotropic because it is required for the synthesis of phospholipids by the liver

In the past ten years many of these observations have been confirmed and extended and several of the new findings may be considered as in line with this interpretation However a number of results have also been reported which have made progressively more complicated the picture and which are not easily reconciled with this interpretation I will try to mention some of these more recent observations with special emphasis on the work which has been or is being done in our laboratory at Bowman Gray

LIPID COMPOSITION OF THE LIVER OF RATS ON LOW PROTEIN DIETS

If the fatty infiltration of the liver of rats fed a low choline diet is due to an impairment in the formation of phospholipids one might expect that the increase in neutral fats is preceded or accompanied by a decrease in the phospholipids of the liver especially in the choline containing fraction

In a systematic study which we carried out for several years (5-11) the lipid composition of the liver of rats on a stock diet was compared with that of rats maintained for various lengths of time on experimental diets with a low content of protein In the liver of the latter animals besides the expected increase in neutral fat a decrease in the level of total phospholipids was uniformly found The decrease was especially marked in the lecithin fraction

with a consequent lower ratio of the choline containing to the total phospholipids When the low protein diets were supplemented with choline, the fatty infiltration of the liver was always easily prevented or reversed but the decrease in the choline containing phospholipids was prevented only under certain experimental conditions

The data in Table I exemplify the differences which were found in this respect when the amounts of fat in the low protein diets were varied It is apparent that the lecithins in the liver were raised to the original level only when the diets contained 20 per cent or more of fat Even with these diets the lecithin values *only* were raised total phospholipids remained more or less below the level found in the liver of animals on the stock diet

From these and other data in the literature it appears that in the liver of rats on low protein diets the fatty infiltration is actually accompanied by a decrease in phospholipids especially in the lecithin fraction However choline administration is much more generally effective in preventing the fatty infiltration th n the changes in the phospholipid level The effectiveness of choline in this respect seems to depend upon additional factors such as the age of the animal the amount of fat ingested and perhaps also the nature of the carbohydrate in the diet When by supplementing the diets with choline an effect on the phospholipid level in the liver is observed this effect is confined almost exclusively to the choline containing fraction Obviously the deficiency in choline or choline precursors is only one and probably not the most important of the factors responsible for the nutritional alterations in animals on low protein diets One may think that the decrease in the total phospholipids of the liver is primarily the expression of a decrease in cytoplasmic material and therefore resembles the changes in the content of ribonucleic acids total proteins and enzymes which become apparent in the liver of animals on low protein diets On the other hand the proportion of the lecithins in the total phospholipids seems to be more directly related to the dietary supply of choline or choline precursors

RATE OF PHOSPHOLIPID SYNTHESIS IN THE LIVER OF CHOLINE DEFICIENT ANIMALS

Attempts to compare the rate of incorporation of P^{32} into the liver lipids of animals on various experimental diets have been made by several investigators inc urselves with results

TABLE I

Effects of Dietary Fat and Choline on the Lipid Composition of the Liver*

Diet	Fat in the Diet	Choline added to the Diet	PHOSPHOLIPIDS			Non phospholipid fatty acids
			Total	Choline-containing		
		%	mg	mg	% of total	
Stock	5		31.5	19.5	62	9
Casein 10%	0-10	0	23.5	12.1	52	135
"	"	0.5	23.7	14.8	62	13
"	20-40	0	23.7	12.7	54	199
"	"	0.5	26.0	20.0	77	34

* Values are the averages of several determinations and are expressed in mg per g of lipid free tissues Reprinted by permission from Fishman W H and Artom C *J Biol Chem* 164, 307 (1946)

which are in substantial agreement(12 13 14) Some of the results of our experiments are illustrated by the data shown in Table II In these experiments young mature rats (10 weeks old) and weanling rats (4 weeks old) were maintained on a stock diet or on experimental diets containing either 25 per cent or 5 per cent casein with respectively a low or a high fat content After periods varying from 1 to 4 weeks on the diets the rats were injected with isotopic phosphate and 6 hours later the radioactivity and the phosphorus were determined in the lipids and in the inorganic phosphate of the liver

In order to minimize the effects of differences in the level of inorganic phosphate all isotopic values have been calculated as a function of the specific activity of the inorganic phosphorus in the liver The values thus calculated have been expressed by three different methods each of these methods is believed to give qualitative information on a definite type of relationship The "relative specific activity" expresses the fraction of the pre-existing phospholipids which have been renewed during the 6 hours following the introduction of the isotope The "relative radioactivity calculated for 1 g. of fat free liver" should represent the activity of a definite weight of tissue in synthesizing phospholipids On the other hand the relative radioactivity referred to a constant body weight should be an indication of the total amounts of phospholipids synthesized by the whole organ during the period of the experiments

From the table it is apparent that in the liver lipids of the animals on the low protein diets the specific activity values were higher than in the groups on the stock or on the high protein diets whereas the radioactivities referred to the whole liver of a 100 g rat or calculated per one gram of the liver were approximately the same in all animals

Substantially identical results were obtained when the rats were maintained on the same diets for longer periods and also in weanling rats The only apparent difference in the results obtained on the older and respectively on the younger rats was that in the latter animals as expected all values tend to be higher than in the older rats on the same diets

A similar constancy in the radioactivity values calculated for the whole liver and for the same body weight has been noted by Flock and Bollman in experiments on rats partially hepatectomized or poisoned with carbon tetrachloride(15) We may conclude there

TABLE II

Incorporation of P^{32} into the Liver Lipid of Rats on Various Diets*

Age of rats	DIET			Liver Fat+	LIVER PHOSPHOLIPIDS		
	Type	Protein	Fat		Relative Specific Activity**	Relative Radioactivity‡ per 1 g fat free tissue	Relative Radioactivity‡ per 100 g body weight
weeks		%	%	mg			
10	Stock	25	5	15	40	0.70	2.78
"	Casein	"	"	28	56	0.79	3.30
"	"	5	"	205	60	0.71	3.22
"	"	"	32	182	71	0.81	3.44
4	Casein	25	5	39	68	0.75	4.30
"	"	5	"	125	106	0.71	4.04
"	"	5	32	238	102	1.00	4.27

\+ mg of neutral fat in 1 g of lipid free liver

** $= \frac{\text{Specific activity lipid P}}{\text{Specific activity inorg P}} \times 100$

‡ $= \frac{\text{Radioactivity lipids}}{\text{Specific activity inorg P}} \times 100$

* Some of these data have been reported in a preliminary report (Artom, C and Cornatzer W E Abstr Commun 1st Intern Congr Biochemistry, Cambridge England p 22) but most data have not been published

fore, that the amounts of phospholipids synthesized in a given time interval by the liver are not markedly affected by protein and choline deficiencies, by partial removal or by toxic damage of the organ In all these conditions the reduction in the functionally active liver is probably compensated by an increased activity of the undamaged cells But the compensation which is apparently adequate for the synthesis of phospholipids does not prevent the occurrence of the fatty liver

In some of our determinations(13) we have separated the liver lipids into choline containing and non choline-containing As shown in Figure 2, the radio activity of the choline containing fraction was practically the same in the liver of animals on the low protein diets as in those on the stock or on a 25 per cent casein diet The only instances in which we found marked decreases in the radioactivity values of the choline containing fraction were those of experiments in which guanidoacetic acid or diethanolamine had been added to the low protein diets As shown by Stetten and Grail(16) several years ago, guanidoacetic acid enhances the fatty infiltration and reduces markedly the lecithin level in the liver of weanling rats on low protein diets These effects have been interpreted as due to a drain of choline in order to make its methyl available for the formation of creatine More recently, we(17) have found that prolonged supplementation of a low protein diet with diethanolamine causes an increase in the total phospholipids of the liver with a considerable decrease in the choline containing fraction Our data led us to believe that diethanolamine is incorporated into the phospholipids of the liver Perhaps these atypical cephalins are metabolized less easily than their natural analogues and therefore accumulate in the liver On the other hand, since choline is probably formed by methylation of ethanolamine, it seems likely that this process would also be impaired if, instead of ethanolamine diethanolamine only were available to the tissue Thus diethanolamine would act as a metabolic antagonist of ethanolamine for the formation of lecithins as well as of natural cephalins

In summary, in intact animals on low protein diets a decrease in the ability of the liver to synthesize phospholipids is not apparent The amounts of choline and ethanolamine available to the liver of these animals seem to be quite sufficient for the formation of phospholipids at a normal rate Since neutral fat accumulates in the liver of these animals, it would appear that the synthesis of phospholipids has a degree of priority higher than other metabolic processes, including the lipotropic effect

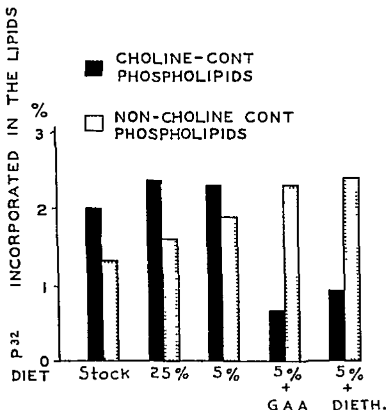


FIGURE 2 The incorporation of isotopic phosphate into the choline-containing and non choline-containing phospholipids of rats on various diets Diets *Stock* containing 25 per cent mixed proteins 25 *per cent* containing 25 per cent purified casein [illegible] GAA same diet [illegible] cent + *Dieth* [illegible] and Cornatzer

To obtain a well detectable reduction in the formation of lecithins one has to superimpose on the dietary restriction of methyl donors the administration of substances which by specific mechanisms further reduce the amounts of choline available to the liver One of the substances tested guanidoacetic acid markedly increases the fatty infiltration of the liver whereas only moderate amounts of fat accumulate in the liver of rats fed diethanolamine This difference is not merely due to a difference in the caloric intake of the two groups of animals since both guanidoacetic acid

and diethanolamine in the amounts used by us caused marked losses of body weight

EFFECTS OF THE ADMINISTRATION OF CHOLINE AND RELATED SUBSTANCES

The original observations of Perlman and Chaikoff that the administration of choline increases the incorporation of P^{32} into the lipids of the liver of rats on low protein high fat diets(4), has been confirmed and extended We have shown that the choline effect occurs even in animals fed low fat diets, but that it is markedly enhanced by the simultaneous administration of a large dose of fat(18)

In all these experiments choline was administered as a single large dose of the compound However, data from several laboratories including our own, indicate that even when choline is added to the diet and therefore ingested in divided doses over a prolonged period a moderate increase in the turnover of phospholipids can still be demonstrated This increase concerns not only the phosphate but also the choline moiety, as it has been shown, several years ago, by Boxer and Stetten in experiments with N^{15} labeled choline(19) The rate of replacement of choline in the tissues of rats on a choline supplemented diet was approximately three times as great as in the period in which the diet did not contain preformed choline

In their early experiments, the Berkeley group had shown that besides choline betaine, and methionine, cystine and cysteine also stimulated the formation of phospholipids(20) The two substances last mentioned not only are not lipotropic but are known to enhance the fatty infiltration of the liver At the International Congress of Physiology held July 1947, at Oxford, England we reported that when a single large dose of ethanolamine was administered to rats on low protein diets, the incorporation of P^{32} into the liver lipids was increased approximately to the same extent as that observed after choline was given(21) Similar observations were published shortly after by Platt and Porter(22)

Several other substances which might be related chemically or biologically to choline were also tested by us(23) In Table III some of the results obtained by others and by us in this respect have been summarized Three comments can be made on this table

First a stimulation of the phospholipid formation in the liver is observed with many N containing compounds, irrespective of the

TABLE III

A Comparison Between the Stimulation of Phospholipid Formation and Other Biological Actions in the Rat*

Substance tested	Incorp of P^{32} in the lipids (liver)	Lipotropic action (liver)	Antihemorrhagic action (kidneys)	Synthesis or replacement of	
				Choline	Ethanolamine
Choline	+	+	+	+	—
Triethylcholine	+	+	+	+	
Betaine	+	+	+	+	—
Methionine	+	+	+	+	
Ethanolamine	+	—	±	+	+
Methylethanolamine	+			+	
Dimethylethanolamine	+	+	±	+	
Diethylethanolamine	+	—	±		
Diethanolamine	+	±			+
Cystine or Cysteine	+	—	—		
DL-serine	—	—			+
Glycine	—	—			+

* Reprinted by permission from Cornatzer W E and Artom C J *Biol Chem* 178 776 (1949)

nitrogen being present as a primary, secondary, or tertiary amine or as a quaternary ammonium Ethyl groups may be totally or partially substituted for the methyls With the exception of ethyl amine, which is not included in the table but seems to possess some activity, all other substances which stimulate the incorporation of P^{32} into the liver lipids, contain a free alcoholic group

Second except for cystine, cysteine, and ethylamine, all of the substances which enhance the incorporation of P^{32} into the liver lipids are either components of natural phospholipids (such as choline and ethanolamine) or precursors of these components (such as methionine, betaine, methyl, and dimethylethanolamine) or they may substitute for these components in the formation of phospholipid analogues

Thus triethylcholine has been demonstrated directly in the tissue phospholipids of animals receiving these compounds We have obtained at least presumptive evidence for the incorporation of diethanolamine into the phospholipids(17) Furthermore we have shown that dimethylethanolamine besides being a probable methyl acceptor for the synthesis of choline, is incorporated directly in the phospholipids even before being further methylated to choline Some of these results are recorded in Table IV(24)

Additional evidence on this point was obtained by us(24 25 26) in which C^{14} labeled dimethylethanolamine was added to liver

TABLE IV

In Vivo* Incorporation of Dimethylethanolamine (DME) into the Phospholipids of Rat Tissue

Substance administered†	DME in Lipid Hydrolyzates	
	Liver	Muscle (10 g)
	micromoles	micromoles
H_2O (1 cc)	0	0
Dimethylethanolamine	70	1
"	90	5
Choline	0	0
Ethanolamine	0	0

† 1 2 millimoles given in one dose by stomach tube 6 hours before killing the rats
* Reprinted by permission from Artom C and Crowder M *Federation Proc* 8 *180 (1949)*

TABLE V*

***In Vitro* Incorporation of Dimethylethanolamine (DME) into the Lipids of Rat Liver Slices†**

Micromoles of substances added to the liver slices	Counts/sec in the lipids (non hydrolyzed)	Counts/sec in the hydrolyzed lipids	
		as DME	as Choline
DME‡ 30	10 745	6 430	694
DME‡ 30, Betaine 20	3 705	1 925	595
DME‡ 30, Methionine 20	6 685	3 340	2 565

† Each flask contained approx 700 mg of tissue in 6 cc medium (Ringer Krebs phosphate pH = 7 4) The flasks were shaken in air or O_2 for 3½ hours at 37° The values are calculated per one gram of moist tissue These data have not been reported

‡ DME labeled in the methyls Approx 1 000 000 counts/sec per 1 g slices were added

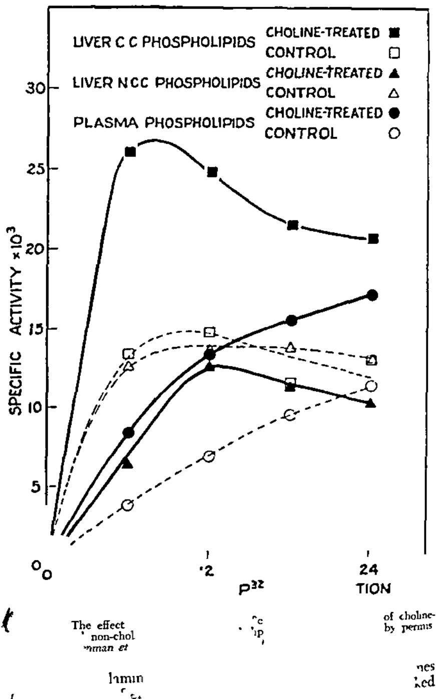
* Crowder M and Artom C Unpublished experiments

slices As shown in Table V quite detectable amounts of the radio activity were found in the lipids and most of it was present in the hydrolytic products as dimethylethanolamine

Third when the biological effects of the various substances are compared with each other it appears in Table III that the lipotropic action has a higher degree of specificity than the stimulation of phospholipid synthesis in the liver Indeed all substances which are lipotropic also cause an increase in the formation of liver phos pholipids but the reverse is not true since several compounds which are not clearly lipotropic do stimulate the incorporation of P^{32} into the liver lipids

The effect of a high dose of choline is chiefly due to an increased formation of lecithins Figure 3 shows some of the findings obtained by Chaikoff and his group in dogs receiving a single large dose of choline(27) It is apparent that the specific activity is increased in the lecithin fraction only and that this increase is often accom panied by a decrease in the specific activity of the non choline containing fraction

In our experiments on rats on low protein diets the absolute values for the radioactivity of both choline containing and non choline-containing phospholipids were always increased after giving

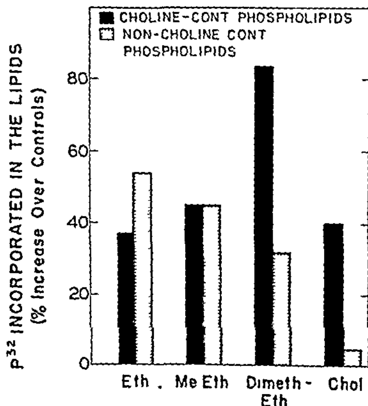


The effect ... non-chol ... of choline- ... by permis

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in the lecithins whereas after ethanolamine was given the effect was often more pronounced in the non choline-containing fraction These points are illustrated in Figure 4

In an attempt to interpret the finding of an increased phospholipid turnover after ethanolamine was given we considered the possibility that in the liver of rats on low protein diets in addition to a deficiency of methyl donors a relative deficiency of methyl



acceptors for the synthesis of choline may also develop The removal of such a limiting factor by the administration of ethanolamine would result in an increased phospholipid turnover, provided that sufficient methyls were available This interpretation is not borne out by the results of experiments in which low protein diets were supplemented with ethanolamine, or with methionine, or with both. When a single large dose of choline, methionine, or ethanolamine were given to the animals each of these substances increased the phospholipid turnover in the liver of all animals, the increases were approximately of the same degree in the groups on the unsupplemented diets as in those on the diets supplemented with the methyl donor or with the methyl acceptor only, or with both(13)

On the basis of these results, it seems unlikely that the stimulation of phospholipid turnover in the liver of animals receiving choline or ethanolamine can be regarded merely as the replacement of a deficiency of methyl donors or methyl acceptors More likely, as suggested by Platt and Porter(22), it is the result of a mass action exerted by the relatively high concentrations of these substances in the liver

Still, there is no doubt that the stimulation of phospholipid turnover is somehow related to the previous diet of the animal since it is small or absent in rats on stock or on high protein diets(13 18) One is perhaps tempted to correlate these differences with the low level of the phospholipids, especially the choline containing fraction, in the liver of the animals on deficient diets Indeed, the most marked effects of the administration of a high dose of choline were observed in the liver lecithins of rats previously maintained on low protein diets containing guanidoacetic acid As mentioned before in the liver of these animals the level of the lecithins is extremely low A comparison of the choline effects on the formation of the phospholipids in the liver of rats on various diets is shown in Figure 5

PLASMA LECITHINS AS A TRANSPORT FORM FOR FATTY ACIDS

If plasma lecithins represent a major transport form for fatty acids, then one could explain the fatty infiltration of the liver as due to an impaired mobilization of fat from the liver of choline deficient animals

In most pathological and physiological hyperlipemias including those occurring during the absorption of fats from the intestine,

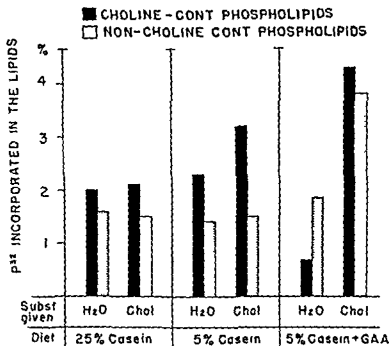


FIGURE 5 A comparison of the effects of a single high dose of choline on the incorporation of isotopic phosphate into the lipids of the liver of rats on various diets From Artom C and Cornatzer W E unpublished results

phospholipids are increased in blood plasma together with the other lipid fraction, and there are some indications that lecithins are chiefly involved in these changes Thus years ago we have shown that in the plasma of rabbits fed large doses of olive oil, the proportion of the lecithins in the total phospholipids increased and that in some instances the choline-containing fraction represented the totality of the plasma phospholipids(29) The more recent determinations by Chaikoff and his group indicate that while in tissues phospholipids consist of a mixture of lecithins, cephalins,

animal

hospho

On the other hand, the gradient of the specific activities of lipid phosphorus in the liver, plasma, and muscle of animals injected with radioactive phosphate had already suggested to Hevesy (31) and to myself(32) that plasma phospholipids originated chiefly or

exclusively in the liver Definitive evidence on this point was obtained by Fishler *et al* (33) who showed that in dogs receiving P^{32} removal of the liver reduces the labeled phospholipids in the plasma to negligible amounts Furthermore in intact dogs injected with radioactive phosphate the administration of a large dose of choline increased the specific activity of the lipid P in plasma as well as in the liver(27)

In a study by Stetten and Salcedo(34) rats were maintained on low protein diets with or without choline supplementation and the body water was enriched with deuterium The amounts of newly formed fatty acids in the depots of the choline deficient rats were the same or less than the amounts found in the controls receiving choline whereas in the liver the reverse was true Assuming that the fatty acids were chiefly synthesized in the liver these findings would be in line with the concept that the administration of choline favors the transfer of the fatty acids from the liver to the depots

The greater ease with which phospholipids are dispersed in water may also suggest that they represent a more suitable form than neutral fats for the transport of fatty acid in blood and for their passage across the wall of the capillaries However the present evidence indicates that in the plasma most or all of the phospholipids are in the form of lipoprotein complexes

Several years ago we studied the filtration of human plasma through collodion membranes of graded permeabilities If the filtration pressure was not too much above that of the blood *in vivo* phospholipids were not found in filtrates containing up to 30 per cent of plasma proteins In other words there was no passage of free phospholipids through membranes which were fairly permeable to plasma proteins and if any splitting of the linkages between proteins and phospholipids did occur this led to a preferential filtration of proteins rather than of phospholipids(35) These were merely model experiments and obviously the extension of the results of such experiments to the permeability of the capillary wall *in vivo* is quite arbitrary However our results may well be compared to the observations of I H Page that the proteins in the urine of nephrotic patients are practically free of phospholipids(36) and with the findings of Man and Peters that in the ascitic fluid and in other transudates the proportion of phospholipids to proteins is the same or lower than in plasma(37)

More direct evidence against the conception that plasma phospholipids represent a major transport form for fatty acids has come

lately from a number of contributions by Chaikoff's group in Berkeley Their results in agreement with previous findings by Hahn and Hevesy(31) suggest that the liver is the main organ not only for the formation but also for the removal of plasma phospholipids When the plasma from a donor dog containing P^{32} labeled phospholipids was injected into eviscerated dogs with intact liver isotopic phospholipids disappeared rapidly from the plasma. Exclusion of the blood supply to the liver in these animals caused an abrupt decrease in the rate of disappearance of the labeled phospholipids from the plasma(38)

A comparison between the specific activity time curves of lipid P in the liver and plasma of dogs receiving choline also shows that the rate of turnover of plasma phospholipids was not increased by choline and that the increases in the specific activity of the plasma phospholipids were merely a reflection of the increased turnover of the lecithins in the liver(39)

Very recently in the same laboratory several of the findings obtained in the experiments with P^{32} have been duplicated by using fatty acids labeled with C^{14}(40) Some of these findings are shown in Figure 6

These results are obviously difficult to reconcile with the hypothesis that plasma phospholipids are a transport form for the fatty acids They rather suggest that there is a continuous rapid exchange of phospholipids between liver and plasma but not between plasma and other tissues Such a difference is perhaps due to the fact that unlike those of most other tissues the cells of the liver are in direct contact with the blood

DOES CHOLINE FAVOR THE CATABOLISM OF FATTY ACIDS IN THE LIVER?

If choline does not promote the mobilization of fatty acids from the liver in the form of plasma phospholipids it seems that one has little choice but that of ascribing the lipotropic effect of choline to an increased catabolism of fatty acids within the liver itself This would bring us back to the idea suggested more than 50 years ago by Loew who wrote that "Lecithins are a machine for burning fats (41)

In mice on a low protein diet with or without added choline Stetten and Grail(42) could not detect any significant difference in the rate at which deuterium containing fatty acids disappear

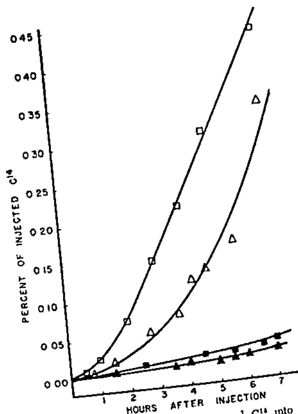


FIGURE 6 The incorporation of injected palmitic acid C^{14} into plasma phospholipids The ordinate represents per cent recovery of injected C^{14} in the phospholipid fatty acids per 100 cc of plasma △ □ normal dogs ▲ ■ hepatectomized dogs Reprinted by permission from Goldman *et al* *J Biol Chem* 184, 727 (1950)

from either depot or liver fat However, differences in rate may be small enough to be within the limits of error of the methods and calculations Still small differences in rate could well account for large increases of fat over a sufficiently prolonged period of time As pointed out by Treadwell *et al* (43) the fat accumulating in the liver of rats on low protein diets is only a little fraction of the total amounts which apparently are utilized or deposited in a normal manner by these animals

Ennor(44) could not detect any change in the oxidation of fatty acids added to slices of livers damaged with phosphorus or carbon tetrachloride However more work with this technique might be worthwhile Indeed, we feel that the *in vitro* experiments might make a notable contribution to this, as well as to other problems of the metabolism of fats and phospholipids

EXPERIMENTS IN THE ISOLATED LIVER TISSUE

In our laboratory we are at present engaged in a study of the formation of phospholipids in liver slices and homogenates Before concluding the present discussion I should like to mention some of the results obtained thus far, since I believe that the comparison of these results with those of the *in vivo* experiments may have some important bearing on an interpretation of the mechanism of the lipotropic action

In a first series of experiments(45) we have studied the incorporation of labeled inorganic phosphate into the phospholipids by liver slices from rats on high, respectively low protein diets The liver from the latter animals was 4 to 8 times less active than slices from rats on a stock diet or on a high casein diet* Supplementation of the low protein diet with choline did not raise appreciably the ability of the liver slices to incorporate P^{32} into the phospholipids These results are illustrated in Table VI When such findings are compared with those of the experiments on intact animals(13), the following explanation for their discrepancy seems plausible In the intact animals phospholipid metabolism is under the influence of regulating mechanisms which maintain its rate at a level adequate

TABLE VI*

Incorporation of Inorganic P^{32} in the Lipids of Liver Slices from Rats on Various Diets†

Exp No	Weeks on Diet	Diet	Dietary Supplement (0 5%)	Per g of Liver Slices: P^{32} Incorporated in the Lipids, micrograms	Per g of Liver Slices: Neutral Fat, mg
21		Stock		16	101
	2	Casein 5%	Guanidoacetic acid	80	28
26	2	Casein 25%		16	137
	"	Casein 5%		59	17
23	4	Casein 5%	Choline	31	30
	"	Casein 5%	Guanidoacetic acid	176	23

† Liver slices incubated for 3½ hours at 37° in O_2

* Reprinted by permission from Artom C, and Swanson, M A *Federation Proc*, 9, 147 (1950)

to the needs of the animals In well fed animals this level is probably much below the maximum functional capacity of the tissue When the animals are placed on a low protein diet, there is a considerable loss of cytoplasmic material and, consequently, also of enzymatic proteins However, because of the large margin in the functional capacity of the liver, the reduction in the amount of enzymes can be easily compensated by an increased activity of the remaining tissue Accordingly, no decrease in the synthesis of phospholipids is detectable in the liver of intact rats on low protein diets On the other hand, in the isolated tissue, where regulating mechanisms are no more effective or less effective, phospholipids are probably turned over at the highest speed compatible with the conditions of the experiments A decreased rate of incorporation of P^{32} into the phospholipids would then be the direct result of the decreased amount of enzyme proteins in the liver of the protein depleted animal

A deficiency of choline in the diet may contribute to these findings, but it is not the primary cause for the decreased ability of the isolated tissue to synthesize phospholipids from inorganic phosphate Indeed, such a decrease cannot be prevented or corrected by adding generous amounts of preformed choline to the diets

Slices of the liver from rats on high, respectively low protein diets seem also to respond differently to the addition of choline *in vitro* As shown in Table VII concentrations of choline which inhibit the incorporation of P^{32} into the lipids of slices from rats on adequate diets tend to stimulate the process in the liver slices from rats on low protein diets There is some similarity between these results, and those obtained in intact animals As mentioned earlier, in these animals the stimulation of phospholipid turnover in the liver after choline administration is clearly demonstrable only when the rats had been previously maintained on low protein diets

In another series of experiments which have just been reported at the last Federation meeting in Cleveland we added C^{14}-labeled choline to liver slices or homogenates(46) When the results of these experiments are compared with those of the previous experiments in which labeled phosphorus was added to liver slices quite marked differences become apparent between the incorporation of the choline and that of the phosphate moiety into the lipids of the isolated tissue Thus, the incorporation of choline was more extensive with slices from rats on low protein diets than with slices of

TABLE VII*

Effects of Choline Added *In Vitro* on the Incorporation of P^{32} into the Lipids of Rat Liver Slices†

Choline added (final concentration)	Rats on high protein diets		Rats on low protein diets	
	No of exper	Average change	No of exper	Average change
moles		per cent		per cent
8×10^{-4}	10	−19	9	+45
3.3×10^{-3}	14	−29	12	+31
7.7×10^{-3}	17	−45	10	+51

† *Increases (+) or decreases (−) percent of the values obtained from the control flasks to which no choline was added All changes are statistically significant* ($P < 0.05$)

* Reprinted by permission from Artom C and Swanson M A *Federation Proc* 9, 147 (1950)

the liver from rats on high protein diets These findings which are summarized in Table VIII are just the opposite of those obtained in the experiments with labeled phosphate

Substitution of nitrogen for oxygen reduces to minimal values the incorporation of isotopic phosphate by liver slices, whereas the incorporation of choline was not decreased, and often was greater in anaerobic conditions Table IX illustrates the differences observed in one experiment in which slices from the same liver were incubated partly with labeled choline and partly with labeled phosphate

TABLE VIII*

Incorporation of Labeled Choline into the Lipids of Liver Slices from Rats on Various Diets†

No of exper	Diet	Neutral fat	Labeled Choline incorporated into the lipids
			γ × 10'
20	Stock	13	139
3	Casein 5%	129	513
14	Casein 5% + G.A.A. 0.5%	135	600

† Slices incubated in O_2 or air for 3½ hours at 37° Average values expressed per 1 g of lipid free tissue The rats were kept on the experimental diets for periods varying between 1 and 8 weeks.

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No of exper	Diet	Neutral fat	Labeled Choline incorporated into the lipids
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3	Casein 5%	129	513
14	Casein 5% + G A A 0 5%	135	600

† Slices incubated in O_2 or air for 3½ hours at 37 Average values expressed per 1 g of lipid free tissue The rats were kept on the experimental diets for periods varying between 1 and 8 weeks

* Reprinted by permission from Artom C et al *Federation Proc* **10**, 157 (1951)

TABLE IX*

A Comparison Between the Incorporation of Labeled Phosphate or Choline into the Lipids of Rat Liver Slices†

Labeled compound	Micrograms incorporated into the lipids	
	In Oxygen	In Nitrogen
H_3PO_4	2 59	0 04
Choline	0 72	4 64

† Rats on Stock Diet Liver slices incubated for 3½ hours at 37° either in O_2 or in N_2

* Reprinted by permission from Artom C *et al* *Federation Proc* 10, 157 (*1951*)

Furthermore as shown by Taurog *et al* (47), homogenization of the tissue suppresses the incorporation of labeled phosphate whereas the incorporation of choline is diminished but still quite appreciable in liver homogenates (Table X)

These results indicate that, at least to a certain extent the formation of the linkage between choline and phosphate may occur independently of the formation of the other ester bonds in the lecithin molecule Apparently, the energy requirements for the incorporation of choline (and probably also of the other nitrogenous constituents of the phospholipids) are much less strict than those necessary for the introduction of inorganic phosphate into the phospholipid molecule This in turn may suggest that the chemical

TABLE X*

A Comparison Between the Incorporation of Labeled Choline into the Lipids of Slices and Homogenates of Rat Liver†

[illegible]	[illegible]	[illegible]	[illegible]
Stock	O	45	25
"	N_2	103	23
Casein 5% + G A A 0 5%	O	317	53
"	N_2	150	75

† Incubated for 3½ hours at 37° in Ringer Krebs-phosphate pH 7 4

* Reprinted by permission from Artom C *et al* *Federation Proc* 10 157 (*1951*)

equilibria involved in the combination between the nitrogenous components and the phosphatidic acid radical may be easily shifted by changes in the concentrations of the reacting substances

The effects of the *in vitro* addition of various substances seem to be in line with this expectation Indeed, if the effect of the isotope dilution is taken into account, choline incorporation in the phospholipids appears to be enhanced by the addition of nonisotopic choline to the flasks This statement is illustrated by the data recorded in Table XI

The effects of the addition to liver slices of various compounds other than choline are summarized in Table XII It is apparent that substances which are natural components of phospholipids (such as ethanolamine or serine) or which may substitute for ethanolamine and choline in the phospholipid molecule (such as triethylcholine, diethanolamine or dimethylethanolamine), when added *in vitro* to the incubated medium, all inhibit the incorporation of choline, probably because they compete with choline for the phosphatidic acid radical The only discrepant results are those of the few experiments in which no effect was observed by thio choline added *in vitro* to liver slices

The extension of results obtained *in vitro* to the conditions of the intact animals is certainly arbitrary However, one might well assume that *in vivo* as well as *in vitro*, the concentration of free choline in the liver cells is an all important factor in determining

TABLE XI*

Effect of Increased Concentration of Non-isotopic Choline on the Incorporation of Labeled Choline into the Lipids of Liver Slices†

Non isotopic choline present in the flask	Isotopic Choline in the Lipids		
	Counts/sec /per gram of liver	Calculated amounts of choline incorporated	
mg		microg	Relative amount
0 24	11 040	8 88	100
0 48	5 700	9 12	103
2 64	2 160	18 48	208

† 3½ hrs incubation in O_2 – Rat on stock diet

* Reprinted by permission from Artom C et al *Federation Proc*, 10, 157 (1951)

TABLE XII*

Effects of Substances added *In Vitro* on the Incorporation of Choline in the Lipids of Rat Liver Slices†

Substance Added (3 3 × 10^{-3} M)	Relative amounts of C^{14} – labeled choline incorporated in the lipids
None	100
Ethanolamine	47
Diethanolamine	32
Methylethanolamine	30
Dimethylethanolamine	36
Triethylcholine	56
Thiocholine	106
Betaine	134 (?)
Serine	74
Glycine	83
Trimethylamine	101

† 3½ hour incubation in O_2 – Rats on stock diet
* Reprinted by permission from Artom C, *et al* *Federation Proc* *10, 157 (1951)*

the rate of the synthesis of lecithins Thus, the inhibition of choline oxidase by fats, noted years ago by Bernheim(48), may result in the maintenance of a greater concentration of choline in the liver and thereby enhance the formation of lecithins Several of the other findings which I have mentioned before could be tentatively interpreted in a similar manner At any rate it is obvious that there is a pressing need for a better knowledge of the metabolic path ways of choline

SUMMARY

In conclusion, while the recent findings *have confirmed* the existence of relationships between the metabolism of phospholipids and the changes in the fat content in the liver, as *affected by lipotropic factors discrepancies have become apparent* in many instances Some of the discrepancies can be ascribed to the existence, in the so called alipotropic diets, of dietary deficiencies other than that of choline or choline precursors Other discrepancies can probably be reconciled by the reasonable assumption that choline

containing phospholipids only are directly involved in fatty acid metabolism, and that, at least to a certain extent the turnover of the nitrogenous constituents can proceed independently of that of the remaining part of the phospholipid molecule

At present it seems more likely that choline acts on the metab olism of fatty acids in the liver itself rather than by promoting their mobilization in the form of plasma phospholipids

Both lecithin formation and fatty acid metabolism probably depend upon the concentration of free choline in the liver cells but there is no conclusive evidence for the hypothesis that the prevention of the fatty infiltration is the result of an increased turnover of liver phospholipids The facts known at present can *be equally well explained by some other assumption such as that* choline is a co enzyme or the precursor of a co-enzyme involved in the oxidation of fatty acids in the liver This hypothetical co enzyme does not necessarily need to be a phospholipid I should like however to mention certain interesting possibilities

Lecithin is an essential constituent of the adenosine triphos phatase obtained from the muscle by Kielley and Meyerhof(49) and this is probably also true for the so called succino oxidase system in muscle and liver(50) On the other hand it is now known that intracellular structures such as the large granules or mitochondria which are very rich in phospholipids contain the enzyme systems for many important oxidation processes including fatty acid oxidation(51)

The lipometabolic phospholipids postulated in the past by R G Sinclair(52) and by myself(2) could well be the lecithins present in the mitochondria as an integral part of enzymes or enzyme systems specifically involved in one or more steps of the oxidation of fatty acids The general relationships between phospholipid turnover and fatty acid metabolism in the liver could thus be explained without postulating that phospholipids actually are inter mediary compounds in the metabolism of fatty acids On the other hand the discrepancies noted in several instances between the accumulation of fatty acids and the rate of phospholipid metab olism determined in the liver as a whole might perhaps be due to the fact that apparently phospholipids are metabolized inde pendently and at different rates in the various intracellular frac tions(53) Only future work will tell if and to what extent these are purely visionary speculations

For the time being, I must conclude this presentation with the obvious confession that we know very little about the mechanism *with which lipotropic agents exert their characteristic action* Still, the efforts of many investigators toward a better understanding of such a mechanism have not been fruitless since their findings have led to notable advances in our knowledge of the metabolism of fatty acids, phospholipids, and choline Moreover, the uncertainties and the obscurities in our interpretations, did not, and should not in the future, discourage our attempt to extend the results of the experiments on animals to the more pressing problems of the diagnosis, prognosis, and treatment of human disease Some of these attempts have been made also in our institution, and the results seem promising I presume Dr Cayer will tell you more about these findings

ACTION OF LIPOTROPIC FACTORS IN MAN

DAVID CAYER

Department of Internal Medicine
Bowman Gray School of Medicine
Wake Forest College

I SHOULD LIKE to extend Dr Artom's observations with our studies on human patients *

By way of correlating what has already been said, liver damage has been produced experimentally in laboratory animals by various procedures, including limitation of the intake of protein, specific amino acids, vitamins, and the use of hepato toxins Although there is some difference of opinion regarding the similarity between the lesions thus obtained in animals and those observed in human cirrhosis, many of the factors which have appeared to act as protective or therapeutic agents in animals have been tried in human beings and found to be of value

The action of choline and methionine in preventing or curing the fatty infiltration of the liver which is produced experimentally in animals by low protein diets is believed to result from an increased turnover of phospholipids in the liver There is a considerable difference of opinion among clinicians as to whether or not lipotropic agents, particularly methionine and choline, have any added value in the treatment of patients with liver disease beyond that which might be expected from the administration of an adequate diet alone But since the liver is the main, if not the total source of phospholipids in the plasma, it would seem that changes in the formation of phospholipids in the liver would be reflected by corresponding changes in the amounts of newly formed phospholipids in the plasma (Figure 7)

Indeed by the use of radioactive phosphorus it has been shown by Drs Artom, Chaikoff and others that the administration of choline or methionine to experimental animals with dietary fatty livers causes an increase in the rate of phospholipid synthesis

We set up a program of study – and our limitations were previously pointed out – of doing multiple liver function tests and

* The investigation reported was supported (in part) by a research grant from the Division of Grants and Fellowships of the National Institutes of Health U S Public Health Service

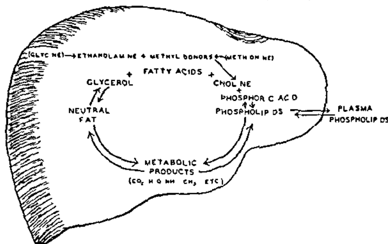


FIGURE 7

needle biopsies All of these patients had evidence of advanced liver disease by biochemical tests and this was confirmed by histologic examination of liver tissue obtained by needle biopsy The patients were injected with a half millicurie of radiophosphorus in the form of sodium phosphate, and 10 Gm of lipotropic material either choline orally or methionine intravenously was given The radioactive and total inorganic phospholipid phosphorus was then determined in 20 cc blood samples at 24 hours and 48 hours, and the results expressed specific activity as well as relative specific activity

Our early studies in normal controls, either medical students or patients who were hospitalized for minor injuries, revealed that in normal individuals the phospholipid turnover, as measured in the plasma varies widely (Figure 8)

In a group of previously untreated patients having portal cirrhosis, the average rate of phospholipid formation was somewhat lower than that of the normal controls, although the individual determinations fell within what we considered to be a normal range (Figure 9) We had hoped that there might be a demonstrable difference between groups but this, of course, was not apparent The specific activity curves showed less variability among these individuals and were more closely grouped than those of the controls

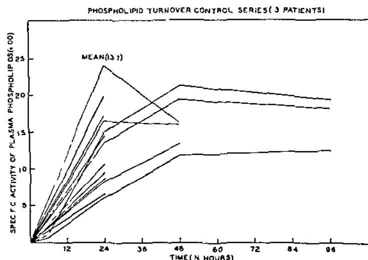


FIGURE 8 Reprinted by permission from *J Clin Investigation* **29,** *534 (1950)*

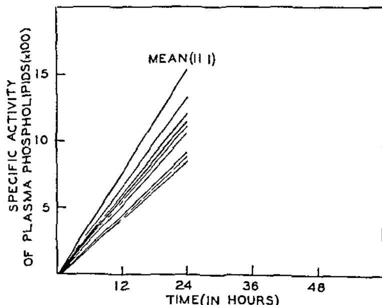


FIGURE 9

When determinations of phospholipid turnover were repeated in these same cirrhotic patients after 8 weeks, or longer of treatment which included lipotropic substances, there was little change in the grouping of the results, even though the patients had apparently made considerable clinical improvement in the interim We could not differentiate, on the basis of the specific activity curves, between the untreated cirrhotic patient and the same patient after treatment when he appeared considerably improved (Figure 10)

The administration of a large dose of lipotropic material after several months of treatment also failed to produce any stimulatory effect The specific activity values were approximately the same after the administration of the single large dose as they were before any test dose or treatment was given (Figure 11)

Although no significant change was obtained after prolonged administration of 3 Gm of methionine or choline per day, it seemed possible that a single large dose of lipotropic material might stimulate phospholipid turnover to a detectable degree Accordingly both normal and cirrhotic patients were studied after receiving a single large dose of lipotropic material Because of the individual variability in both the level of phospholipid phosphorus and phospholipid formation, it was felt that the same individual should be used as his own control in order to detect any significant change which might occur as the result of the administration of the lipotropic substance

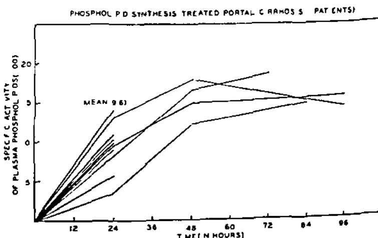


Figure 10

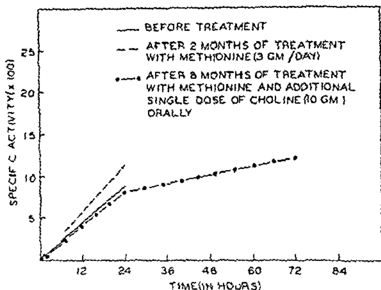


FIGURE 11 Reprinted by permission from Cayer D and Cornatzer W E So th M J 42 669 (1949)

The rate of phospholipid turnover as measured in the plasma in a normal individual in spite of the variability between individuals remains fairly constant for the same person over periods up to 6 months We found no significant difference in the phospholipid levels following a single large dose of choline or methionine The specific activity time curve remains essentially unchanged (Figure 12)

In experimental animals receiving a high fat low choline diet the injection of a single large dose of choline does have a marked stimulatory effect on the conversion of inorganic phosphate into phospholipids In animals on adequate diets containing an abundant supply of choline or choline precursors this effect is not apparent If the effects of choline in human beings are analogous to those in animals our results obtained in persons without clinical or laboratory evidence of liver disease would indicate that their dietary supply of lipotropic substances was adequate

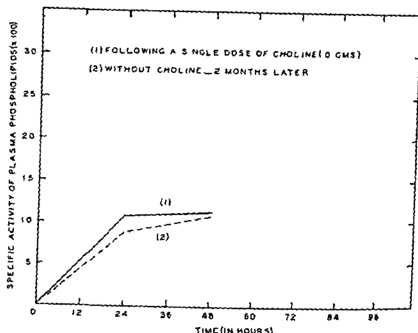


FIGURE 12

We then studied 20 consecutive hospitalized patients All had clinical and laboratory evidence of advanced liver disease, but were otherwise unselected There were 17 males and 3 females The youngest was 10 years of age, the oldest 56, the average 39 7

At the time of hospitalization, all the patients had enlarged or palpable livers, 11 had demonstrable ascites, and 4 were jaundiced The spleen was palpable in 10 patients None of these patients had received any previous therapy with vitamins or lipotropic material All had definite impairment of liver function as determined by accessory biochemical tests and the clinical diagnosis was confirmed in each instance by histologic study of the biopsy material It is of interest that of the 20 patients studied 10 had evidence of fatty infiltration demonstrable by examination of the biopsy material All of these patients remained in the hospital for at least 8 weeks

Here are cases illustrative of the findings in each group

This patient at the time of admission to the hospital had marked enlargement of the liver After 2 months of treatment her liver regressed considerably in size (Figure 13)

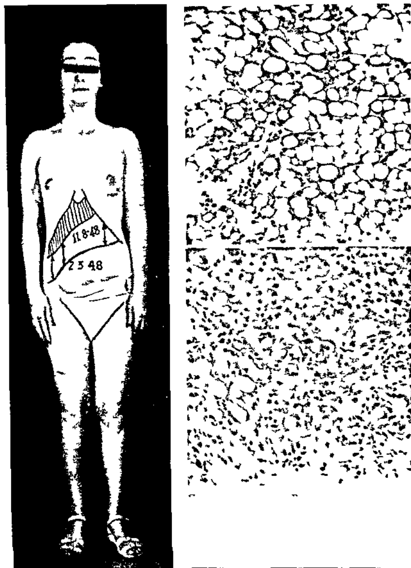


FIGURE 13 Thirty six year old woman with hepatomegaly and fatty metamorphosis showing regression in liver size during interval of study

Figure 14 represents the first biopsy of this patient's liver The remarkable fact as already noted by Dr Artom and others is that a liver which is thus infiltrated with fat and obviously must have undergone a marked metabolic disturbance still continues to make phospholipids Figure 15 represents a repeat liver biopsy after an 8 week interval There is still some inflammation and fat glob les but it has resolved in a remarkable fashion Of course this has been noted by other observers as well

The phospholipid turnover curve at the onset of treatment when the large 10 Gm dose of methionine was given was compared with that following 2 months of treatment during which the patient had the usual dietary regimen in addition to 3 0 g of methionine a day It showed the rate of phospholipid turnover as measured in the plasma to be significantly altered In other words an increased rate was obtained when the large dose of lipotropic material was given at the time the liver contained fat (Figure 16)

We learned quickly that we could not differentiate on the basis of clinical examination or routine laboratory studies and liver function tests whether or not these patients with enlarged livers would have fatty or badly scarred livers with marked inflammatory change

Figure 17 shows a patient with marked enlargement of the liver who also had a regression in the size of the liver after a two month interval of treatment

Biopsy of this patient's liver showed no fat but showed marked scarring and some degenerative changes (Figure 18)

Watson Did you stain that for fat?

Cayer No sir We have in all these biopsies interpreted the vacuoles as indicative of fat

The repeat biopsy after 2 months of treatment shows little change except possibly some increase in fibrosis (Figure 19) We might have suspected this clinically since the liver regressed in size

The phospholipid turnover was determined on this patient at the onset and after 8 weeks of treatment on each occasion with a large test dose of lipotropic material in this instance methionine You will notice that this patient responds pretty much as does the normal control The rates of phospholipid turnover were unchanged We might have anticipated that since no fat was

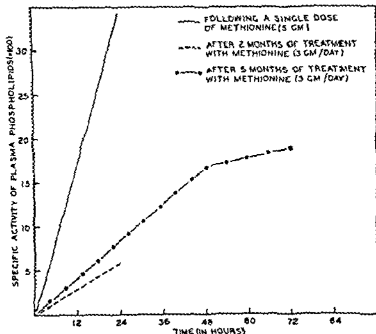


FIGURE 16 Rate of phospholipid turnover before and after eight and twenty weeks of treatment Note increased turnover when biopsy showed marked fatty change Reprinted by permission from Cayer D and Cornatzer W E *Science* 109, 613 (1949)

present in the liver, that he would not respond, and such was the case (Figure 20)

Ten of the 20 patients studied had fat Of these, 9 showed a significant increase in the rate of phospholipid turnover as measured in the plasma following a 10 Gm dose of lipotropic material at the onset of treatment One patient who had marked fatty infiltration of the liver showed no such response This patient expired We wondered whether he had reached a metabolic impasse when he could no longer respond with an increased rate of turnover after a large dose of lipotropic material It suggests that the phospholipid response of the cirrhotic patient who has fatty infiltration of the liver may also provide a clue as to his ultimate prognosis

Of 10 patients who had only inflammatory and fibrotic changes in the liver 8 failed to show an increase in the rate of phospho-

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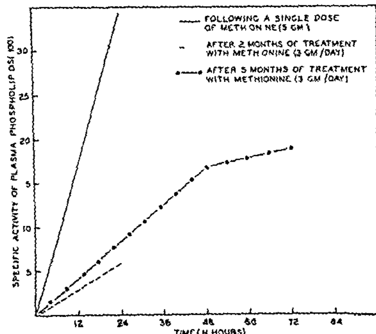
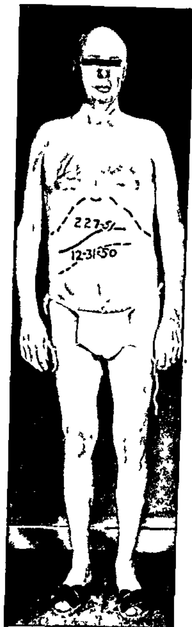


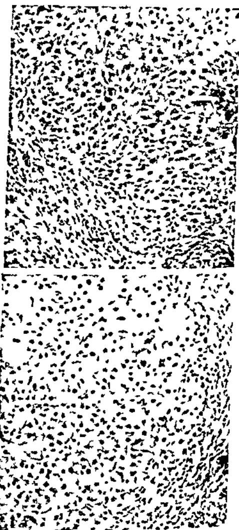
FIGURE 16 Rate of phospholipid turnover before and after eight and twenty weeks of treatment Note increased turnover when biopsy showed marked fatty change Reprinted by permission from Cayer D and Cornatzer W E *Science* **109** *613 (1949)*

present in the liver that he would not respond and such was the case (Figure 20)

Ten of the 20 patients studied had fat Of these 9 showed a significant increase in the rate of phospholipid turnover as measured in the plasma following a 10 Gm dose of lipotropic material at the onset of treatment One patient who had marked fatty infiltration of the liver showed no such response This patient expired We wondered whether he had reached a metabolic impasse when he could no longer respond with an increased rate of turnover after a large dose of lipotropic material It suggests that the phospholipid response of the cirrhotic patient who has fatty infiltration of the liver may also provide a clue as to his ultimate prognosis

Of 10 patients who had only inflammatory and fibrotic changes in the liver 8 failed to show an increase in the rate of phospho





FIGURES 18 AND 19 Biopsies taken on admission and after eight weeks of therapy showing no change other than increased fibrosis

FIGURE 17 Forty eight year old man with hepatomegally Regression in liver size during eight week period of study probably due to contraction of scar tissue

PHOSPHOLIPID TURNOVER IN CHRONIC HEPATITIS WITH NO FATTY INFILTRATION

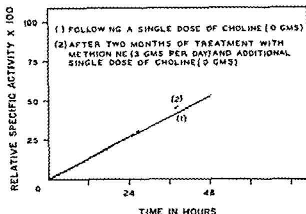


FIGURE 20 Rate of phospholipid turnover with single large dose of lipotropic material before and after eight weeks of treatment No response to choline occurred when no fatty change was present

lipid turnover when the initial curve was compared with that obtained after an 8 week interval of treatment Two of those patients however did show some increase in the rate of phospholipid turnover even though by the staining method used there was no demonstrable fat in the liver

Watson Dr Cayer would you mind commenting on the history of those two groups of 10 patients with respect to alcoholism and with respect to diet?

Cayer The histories are almost identical in this respect Dr Watson

Of the 20 patients 15 by history were marked alcoholics One was a boy of 10 He had no such history In the four others a history of drinking was obtained but from the history alone it could not be said that it was excessive I would say that in all of these patients there was evidence by history that they had been eating diets low in protein high in carbohydrate and fat

Watson Was there any difference in the lower group in the ones in which the biopsy failed to show fat in the interval from

the last drinking? In other words, were they sicker individuals who had gone a longer period without alcohol, or was there any difference in that respect?

Cayer No, sir, I would say – and again I cannot be too definite about this – that the patients who had been drinking at the time of admission were usually those that showed fat

Watson Yes, that is what I meant with respect to that lower group – whether they had gone a longer period without drinking

That has been our experience, too that when they have been drinking right up to the time of admission or within a short time of admission, they have a large amount of fat If they have gone for, let's say, two months without drinking the fat often has diminished or even disappeared from the liver by the time we get to see them

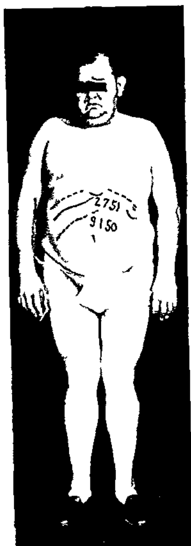
Best You could reproduce that very easily in animals Dr Hartroft and I were talking about that today You could get a cirrhotic liver with a lot of fat and then put the animals back on a diet rich in lipotropic factors and the fat would disappear quickly leaving the fibrosis

Watson That is right

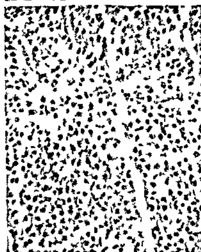
Cayer There is a question on which clinicians differ, namely Is there any value to the administration of a large dose of lipotropic material to these patients at the onset of their illness? I think we can say from our study that after 8 weeks there is no detectable change in phospholipid synthesis We would interpret this as meaning that, if lipotropic agents are of value in the treatment of patients with fatty livers the effect is limited In all probability if such a patient can eat, the result over a period of a month will be essentially the same with or without the added administration of lipotropic factors We have felt, from observation of two instances where patients were admitted in coma with marked fatty infiltration of the liver, that the administration of lipotropic agents at that particular time may actually have been life saving

The same question exists as to whether or not these lipotropic substances are of value in treating infectious hepatitis

We have studied 7 patients with infectious hepatitis One, a man who had bleeding hemorrhoids, for which he had received four or five transfusions about 8 weeks before he was admitted to







FIGURES 22 AND 23 Biopsies taken on admission and after five months of therapy showing resolution of inflammatory changes Reprinted by permission from Caver D The use of lipotropic factors in the treatment of liver disease *Gastroenterology* (in press)

FIGURE 21 Thirty one year old man with hepatomegaly due to homologous serum hepatitis

the hospital for hemorrhoidectomy At the time he came in he was jaundiced, still severely anemic, and had marked enlargement of his liver (Figure 21)

The admission biopsy was compatible with the clinical diagnosis of homologous serum hepatitis (Figure 22)

This man did not return to us until 5 months after the time of his discharge At his second admission his liver function tests were essentially normal The repeat biopsy showed resolution of the inflammatory changes (Figure 23)

The curves for phospholipid turnover showed no increase in rate following a large dose of lipotropic material (Figure 24) In this respect, in spite of the marked inflammatory change in the liver, the patient with infectious hepatitis responds pretty much as does the normal individual

In conclusion, then we can say that in normal individuals the rate of phospholipid turnover remains constant over study periods up to 6 months, and is uninfluenced by the administration of large amounts of lipotropic material when 10 Gm of choline or methionine are given to cirrhotic patients whose liver biopsy shows fatty infiltration, the rate of phospholipid turnover at the onset of treatment exceeds that found later when the liver contains less fat or none at all in cirrhotic patients whose liver biopsy shows necrosis, inflammation or scarring without fat the rate of phospholipid turnover is not stimulated by choline or methionine

We have seen a repetition of this experiment where patients who show a high rate of phospholipid turnover when they have 3 or 4 plus liver fat, are restudied 2 months later when the liver shows only 1 plus fat, and have a decrease in the rate of turnover then resume their drinking are rehospitalized again showing increased fatty infiltration, at which time the repeat curve of phospholipid turnover her than it ie time of the first discharge fro tal

All the biocl ies were c cone not familiar with the clinic on these pathologist who interpreted the ns saw n tients When the attempt was m te the a correlation en the his s and sma phos d turnove age ents One who had f respond

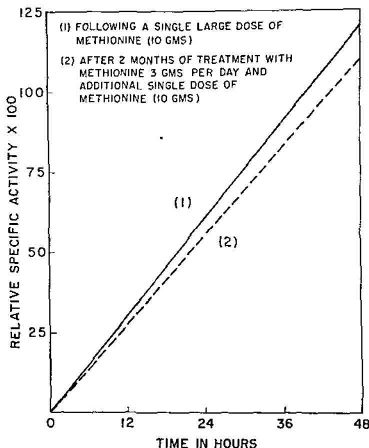


FIGURE 24 Rate of phospholipid turnover with single large dose of lipotropic material before and after five months of treatment Turnover unchanged by infectious process of large dose of methionine Reprinted by permission from Cayer, D The use of lipotropic factors in the treatment of liver disease *Gastroenterology (in press)*

was the only patient who expired We find some difficulty in explaining why two patients with inflammatory and fibrotic changes in the liver still showed a response to a large dose of lipotropic material There is the remote possibility that the biopsy obtained was not representative of the changes in the liver, or perhaps that some short interval before these patients were studied they d

have fat At the time we saw them, fat was not histologically demonstrable, although the response to the lipotropic material was still present

Best The papers by Dr Artom and Dr Cayer are open for discussion

Stetten I should like to ask Dr Cayer one question When methionine or choline was administered to patients whose liver biopsy had shown a great deal of fat was a lipemia noted to succeed the administration of the lipotropic agent?

Cayer Dr Stetten, I cannot answer that We did not determine total lipids

Sherlock Can you tell me whether you have any evidence of increased phospholipid turnover in other conditions which cause fatty changes in the liver, such as diabetes or obesity?

Cayer We have studied other patients with enlarged livers In a few diabetics, fatty infiltration of the liver was present We have not evaluated the data in these patients, however

Sherlock But it was increased?

Cayer It appeared to be increased

I should mention that it was of some concern to us during the study whether or not the half millicurie dose of radio phosphorus might potentially be the straw that breaks the camel's back in a patient critically ill with liver disease So far as we could tell there were no harmful effects even in the comatose patients who had received phosphorus At the end of 8 weeks, when the studies were repeated, there was no longer any evidence of radioactivity in the patients

Dr Hanger mentioned one patient with marked enlargement of the liver who responded to choline with a rapid regression in size of the liver We also have seen this and even suspected that the patient perhaps was undergoing subacute or acute yellow atrophy perhaps related to the injection of phosphorus But the fact that the patient got well and did so rapidly apparently meant that the marked decrease in size was due to the rapid removal of fat from the liver

Best Of course, it is extremely difficult to coordinate the experimental results and the clinical findings I think one is on the safe

side to keep on repeating that the only thing that is likely to respond to the administration of choline is choline deficiency

Patek Mr Chairman I would say that in clinical practice for every case cited of dramatic reduction in size of the liver, I think one could find an equal number with no response to the administration of choline This may be another way of saying what you just said Mr Chairman — that there are those with choline deficiency and those who are perhaps deficient in some other substance as well

Experimentally we(54) have been doing some work with rats which might support the idea that there may be another factor or factors involved Rats were placed on a low protein cirrhosis producing diet *In line with Dr Artom s remarks* this diet doubtless has multiple deficiencies Cirrhosis was produced in 3 series of rats on a diet that was patterned after Dr Sebrell s diet containing 4 per cent casein Liver biopsies were performed after 16 weeks

There was considerable variability in the degree and extent of cirrhosis — as has been the experience of others After grouping *the rats according to the histological severity of the lesions* we placed them on 3 dietary regimens One group receiving the 4 per cent casein diet with added choline (40 mgm daily) showed a sharp reduction in mortality but did poorly nutritionally and clinically At the end of 16 weeks these animals were sacrificed The livers still showed considerable fat and if anything progression of fibrous tissue when compared to the biopsy

A second group was given the 4 per cent casein diet with added choline and also methionine in amounts equivalent to a 30 per cent casein diet This group performed similarly to the first group

A third series received 30 per cent casein diet and these rats not only showed much better clinical improvement but their livers also showed greater reparative changes with loss of fat and some decrease in fibrous tissue

So it appeared that casein may have provided other factors beside choline which exerted a therapeutic effect on fat content liver cell regeneration and resorption of connective tissue

This supports certain of the more recent observations of Dr Gyorgy(55) who found that in moderate degrees of experimental cirrhosis one obtained beneficial results from choline and methio

nine but in those rats with severe cirrhosis there was no manifest improvement If one can interpret this I think it indicates that choline and methionine show maximal reparative effect in the early stages of cirrhosis (as shown with nutritional cirrhosis by Dr Sebrell and his associates(56) and with CCl_4 cirrhosis by Dr Best and his associates(57) If the experiment is extended over a longer period of time — with more severe cirrhosis — other deficiencies may become apparent so that the beneficial effects of choline and methionine are more or less lost It suggests that additional dietary substances in casein possibly amino acids may be necessary for the repair of the liver

Gyorgy Dr Patek did you reverse your experiment and start with 4 per cent protein which is obviously exceedingly low and give choline from the beginning as a prophylactic measure?

I think it is easy to make a logical mistake by drawing a conclusion from therapeutic experiments with regard to prevention — treatment versus prevention You may prevent with choline but you will not necessarily cure the pathological condition in question

Patek That is right Prevention and treatment are certainly different problems We had one control group with 4 per cent casein and added choline (from the start of the experiment) which maintained fair health as I recall

Gyorgy That is important

Patek That is the difference

Gyorgy Therefore I think what you have is an unresponsive target organ

Patek Well it responds to the casein perfectly well

Gyorgy Oh yes because with casein as complete protein you build up the enzymes which are already now depleted in the disease

Patek That may be Well then it is something more than choline

Gyorgy What we cannot do is to confuse therapy with prevention

Patek We agree

Best Obviously the better experiment if you are studying the effect of choline would be to give a diet adequate in all the amino acids

Patek But deficient in choline

Best Yes Of course, the methionine has been the trouble all along in that you just have to compromise and give some methionine but not too much I don't know of any way of getting around the difficulty

Gyorgy Well, you can give peanut meal, in a very high protein diet

Best I know, but you cannot give a diet low in choline precursors

Gyorgy With peanut meal you get the most severe cirrhosis and fatty liver

Artom Dr Best, one way to get around the difficulty you are mentioning may be by the use of substances such as guanidoacetic acid or diethanolamine which are supposed to act specifically on the metabolism of choline (either by enhancing its destruction or by interfering with its synthesis) While the interpretation of the results may be somewhat uncertain I believe that there is still much to be done with these and other metabolic antagonists or analogues of choline and of its precursors

Popper I would like to mention that ethionine an analogue and antagonist of methionine produces in fasting female rats (within 48 hours), fatty metamorphosis of the liver(58) which cannot be prevented by administration of choline but of methionine

Best That is probably a toxic fatty liver isn't it?

Popper I do not think so sir because the fatty liver can be prevented by stoichiometric doses of methionine In addition, studies by Koch Weser and Farber(59) in our laboratory failed to show impairment of liver function in these animals with the exception of bromsulfalein retention and slight serum bilirubin elevation The latter changes were explained as a circulatory effect produced by compression of the sinusoids by fat loaded liver cells In addition recent enzyme studies of Koch Weser and Huerga have shown that only the hepatic choline oxidase of a large number of enzymes examined in liver and serum decreases in acute ethionine intoxication

Artom I would like to mention a report from Chaikoff's laboratory concerning the destruction of the acinar tissue of the pancreas of rats receiving ethionine(60) The microphotographs are sugges

tive of a sort of acute pancreatic necrosis with the islets intact Of course the authors think of a possible correlation between the fatty liver and the deficiency of the external secretion of the pancreas Such a correlation would be similar to that described by these workers for the fatty infiltration of the liver following ligation of the pancreatic ducts in dogs

Popper Farber in our laboratory had shown the same(61) This pancreatic necrosis also does not respond to choline but can be prevented by methionine It occurs in contrast to the fatty liver in male as well as in female rats fasted or not fasted The presentation of Davies at the last Liver Injury meeting(62) stimulated us to perform these studies by acquainting us with the fact that the pancreas is more sensitive to protein deficiency than is the liver As far as the fatty liver is concerned Farber and Koch Weser(63) demonstrated a protective effect of the male sex hormone they found that males lost their resistance to ethionine after castration and that testosterone propionate protected both females and castrated males In ethionine treated males and females lesions of the excretory parenchyma of the pancreas develop equally They start with a loss of basophilia of the epithelial cells followed within 48 hours by necrosis and inflammatory changes and on the 4th day fatty necrosis may be noted Dr M Grossman in Dr Ivy s laboratory has to my knowledge observed similar changes in dogs after ethionine administration and was also able to produce chronic lesions

Best Any other comments?

Hill I was quite interested in the remarks about one patient that had fatty liver infiltration and was put on choline and died I presume there is assumed a direct connection between the two because it is quite well known that children with fatty liver who are put on a high protein diet tend to react very badly and tend to die I know in Curacao West Indies they have a condition there which shows fatty metamorphosis of the liver and incidentally Hartz(64) claims it never goes to cirrhosis *When these children* come in if they are put on Mead Johnson s milk they become very very ill The only way you can get them well is to put them on quarter strength then half strength and then build them up

In Jamaica we do not have much fatty liver disease Only 5 per cent of our cases show fatty metamorphosis They have some additional underlying pathology which I shall tell you about tomorrow

But the cases that have fatty metamorphosis do not want to eat They are quiet they are sick and they just simply refuse food We put two or three on a high protein diet one nearly died and the others became seriously ill We have got to nurse them gradually It looks as though with fatty metamorphosis the liver just cannot take the high protein in the diet

The other interesting thing is that the majority of our cases (those who do not show fatty metamorphosis) have voracious appetites but they do not want cereals They love proteins They literally lap them up They will eat anything you give them except cereals

Watson What group?

Hill The ones that do not show fatty metamorphosis actually 65 per cent of our cases

Watson Just malnourished

Turner What about the liver?

Hill We can show a condition of collagenosis going on to fibrosis

It is interesting that the cases with fatty metamorphosis have a low protein and a low calorific diet whereas the majority of our cases in the West Indies have a low protein and a normal or high calorific diet

I was interested to hear about the alcoholics who had a high calorific diet Our children — and their ages varied from 4 months to 16 years the majority studied were under 4 years of age — have had low normal and high calorific diets and it has been the low calorific diets that have produced fatty metamorphosis

The other comment I should like to make is on the use of lipotropic choline Well it is so Choline is lipotropic We know that But I don t like the term because it strikes me that this fatty metamorphosis in the condition in which we find it in the Tropics I would liken to the running nose and a "cold" The running nose part is the fatty metamorphosis and the cold, part is perhaps the liver cirrhosis And to my mind there is about as much relation between the two as there is between the running nose and a cold" The running nose is only a symptom of the cold not the cause of it likewise fatty metamorphosis and liver disease

The very fact that choline works sometimes and sometimes it doesn t and that methionine works sometimes and sometimes it

doesn't, surely shows that there is something else fundamental going on It is not the lipotropic action which is fundamental it is some other mechanism

We have been using some blunderbuss therapy down in Jamaica We have not got your choline and methionine, but we have been giving our patients high protein diets, and our children have actually reacted on these high protein diets after 2 to 4 months The liver has regressed and so on

I did talk about blunderbuss therapy We put some of our children on Ventriculin®, and the results in 13 out of 16 cases have been most remarkable both in cirrhosis and in a condition I shall describe tomorrow One case, for example, reacted in 4 days, and all of them reacted within 3 weeks A liver which had been 5 fingers' breadth below the costal margin, regressed, and it was mentioned here that that regression might have been due to the disappearance of fat There was not a bit of it in biopsies which were carefully followed up There was no fat at all in the liver The regression was due to something else

So what I am trying to say is that with regard to the use of lipotropic choline,' I wish you would use the term "choline" and leave the lipotropic" action out We do know it is lipotropic, but it rather begs the question for a person like me It gives me a fixed idea – a fixation – about what is happening in this condition

Best How could we oblige? I don't know just what we could do here

Gyorgy I should like to ask Dr Artom, does the intestinal mucosa play any part in the effect of choline, and do you have any increased or improved absorption or synthesis of lipids in the intestinal mucosa and could that be a little obscured by working only on the liver slices?

Artom Some time ago, we studied the incorporation of P^{32} in the phospholipids of the small intestine, using rats on low protein diet(65) Our results proved definitely, at least in my opinion, that in these animals choline stimulates the turnover of the phospholipids in the intestinal mucosa just as it does in the liver, and that when fat is given at the same time, the choline effect is enhanced just as it is in the liver These findings indicate that there is a relationship between the absorption of fats and the metabolism of phospholipids in the intestinal mucosa They are even suggestive

of the possibility that the supply of choline to the intestinal mucosa may represent a limiting factor for the absorption of fats But there is a point which I would like to stress here again that these data do not necessarily prove that phospholipids are directly involved as intermediary products in the absorption of fats

I have made substantially the same reasoning on other occasions, for instance, when I discussed Dr Verzár's results(66) many years ago, on the inhibition of fat absorption by iodoacetate or phlorizin in relation to our own experiments with radioactive phosphorus These showed an increased incorporation of P^{32} in the phospho lipids of the intestine of rats after the ingestion of fat I said then that both groups of findings could be explained by assuming that the absorption of fats involves an increased metabolism of all the constituents of the cells, including the phospholipids If so, an impairment or limitation in the synthesis of any of these constituents might result in an impairment or in a limitation of the absorption of fats

Gyorgy Would it not be possible that you have increased phospholipid synthesis in the intestinal mucosa and the liver cannot take them up, because it is already filled with fat In your patients with fatty liver, Dr Cayer, when you gave choline or methionine you might have increased the phospholipid synthesis in the intestinal mucosa and the phospholipids bypass the liver, and therefore you may get higher values in the plasma

Artom I believe that this is a distinct possibility I think that Dr Bollman has shown that there is an increase in the phospho lipids of the intestinal lymph during the absorption of fat

Bollman That is true

Artom This increase should contribute something to the plasma phospholipids

Bollman In our studies of intestinal lymph(67a,b) it was evident that even in the fasting animal phospholipid was being added to the blood by the intestinal lymph With the feeding of fat there was considerable increase in the phospholipid content of the lymph from the intestine which would be entering the blood had we not made the lymphatic fistula Experiments with P^{32} indicated that much of the phospholipid was newly formed in the intestine. So here we are at a little point of difference from Chaikoff's experiments indicating that the liver is the sole source of plasma pho-

lipids We have repeated his experiments with the hepatectomized animal and have obtained the same results

Gyorgy What do you mean the same results ?

Bollman The same results that Fishler in Chaikoff's laboratory (33) found that is after giving P^{32} the labeled phospholipid does not increase in the plasma of the hepatectomized animal We have not fed the hepatectomized animals for this experiment because in the process of hepatectomy we have interfered greatly with intestinal absorption and perhaps damaged many lymphatics I am not sure that we would get anything but negative results if we did the experiment But certainly there is a source for some plasma phospholipids from the intestinal mucosa

Best Surely you have interfered completely with absorption haven t you with complete removal of the liver?

Bollman Not entirely Some substances may be absorbed but the quantitative function is depressed The circulation from the intestine is taken care of by the portal vein and the portal blood returns to the heart via the collateral circulation which had been developed by the preliminary operations

Best I see

Bollman The histologic condition of the intestine appears normal but functionally the motility is depressed following the laparotomy and absorption is delayed so that even very soluble materials remain in the intestine much longer than in the normal animal

Watson Do those observations in any way alter your calculations with respect to the increased phospholipid turnover in those cases of primary biliary cirrhosis or cholangitis that you and Dr Balfour studied?

Bollman No because Balfour and I only studied the phospholipid turnover in the blood with no reference as to its source(68)

Watson Well you assumed that that was all related to the liver

Bollman Well no as a matter of fact it did not seem to be directly related to the liver

Watson I thought that you had the impression that it was an overproduction in the liver

Bollman No all that we could decide was that those patients with high levels of phospholipid in the plasma also had a high rate of

phospholipid turnover Different rates of plasma phospholipid turn over did not correlate with other functional evaluation of the liver We do know that other organs make phospholipid at normal rates even in the absence of the liver and the only evidence of the control of plasma phospholipids is the failure of P^{32} incorporation in the plasma phospholipids after removal of the liver

Gyorgy Therefore, you agree that there is a synthesis in the intestinal mucosa?

Bollman Oh yes very definitely However I do not know whether it is a major or a minor factor in supplying phospholipid to the plasma The plasma phospholipid does not decrease appreciably with continuous drainage of intestinal lymph nor does it decrease after removal of the liver

Gyorgy Could you not study this in Eck fistula dogs?

Bollman One could but there would be many complicating factors which would to my mind make interpretation of the results almost impossible

Artom Don't you think there are so many collateral paths that it could reach the liver even with an Eck fistula?

Madden Is there some method of distinguishing between incorporation of radioactive phosphorus into phospholipid and new synthesis of phospholipid, both in liver and intestinal mucosa? What about radiophosphorus incorporation as compared with choline incorporation as a measure of phospholipid production? These don't seem to be parallel in Dr Artom's data How significant are they?

And also how much overlap is there between phospholipid and lipoprotein, how much of lipoprotein is phospholipid? Could there be a limiting or an accelerating phenomenon contributed by the necessity for a protein element of whatever it is that is synthesized in the liver and appears in the plasma as phospholipid?

Artom I think there is some relationship but it is hard to put your finger on it I am sure that the formation of the ester bond between the nitrogenous component and the rest of the molecule is relatively independent of the incorporation of the phosphate part, which is in the middle of the phospholipid molecule But it seems to me that it would not be easy to show these differences *in vivo*, because I think that *in vivo*, normally the various parts of

the phospholipid molecule are metabolized more or less at the same rate You have to find some condition in which artificially you can separate the synthesis of the various bonds This is probably what occurs in the *in vitro* experiments

Madden It just occurs to me that this might explain some of the difference between the *in vitro* and *in vivo* observations the possibility that *in vitro* semiabnormal lipoprotein is produced with maybe relatively normal phospholipid production

Artom Yes I think it is quite possible

Madden Therefore the apparent rates might be rather different

Turner We have had a small experience in studying the labeling of the different parts of our centrifugate with radioactive phosphorus and the time factors are very interesting We have followed our individuals over a period of 7 days after the injection of the phosphorus One part of the centrifugate would be labeled before another

We don t know how to interpret these findings except that they encourage us to believe that the phospholipids at different portions of our column of centrifugate are functionally different entities Why this lag before labeling is found in some portions of the centrifugate is an extremely puzzling thing

Hanger Which part becomes labeled first?

Turner The centrifugate in the zone of high medium density most frequently

Best As I understand it Dr Turner you might have 10 per cent of the phosphorus in the No 1 fraction labeled and 5 per cent of the total in another – I mean different proportions of the total phosphorus labeled in the different fractions

Turner Yes

Best That is extremely interesting

Artom I have mentioned a while ago a paper by Ada which appeared in 1949 in the *Biochemical Journal*(53) This author has studied the rate of incorporation of P^{32} into the phospholipids of the various fractions of the liver separated by differential centrifugation The impression one gets from reading his curves is that in each fraction the synthesis of phospholipid is more or less independent from that occurring in the other fractions When these

curves are compared with the curve obtained for the phospholipids in blood plasma, the only fraction which could possibly contain the precursor of the plasma phospholipids is that of the large granules of the liver In other words, the phospholipids of the liver mitochondria might be the immediate precursor of the phospholipids in plasma

Shorr Dr Artom do you know what percentage of the mitochondria is phosphorus? Actually, the mitochondria represents almost a third of the cell volume, doesn't it?

Artom I think so We have done several analyses of the mito chondria of rat liver(69) There is something like 30 per cent of the dry weight made of lipids, of which 75 or 80 per cent are phospholipids

Shorr And about a ninth of the cell volume

Artom About a tenth of the cell volume The rest of the ma terials which compose the mitochondria are proteins and nucleoproteins So actually, mitochondria are quite rich in substances which look like phospholipids The proportion however, of the choline containing and non choline containing phospholipids is ap proximately the same as in the unfractional liver So while there are more lipids and more phospholipids in the mitochondria then in the liver taken as a whole it does not seem that there are or, at least, we were not able to detect any marked differences in regard to the composition of these lipid mixtures We could not find peculiar lipids such as cerebrosides or lysophospholipids which have been suggested in the past as major components of the mito chondria If actually large proportions of these peculiar lipids exist in the liver, they must be concentrated in some other fraction, not in the mitochondria

Stetten You have pointed out an apparent conflict in the picture Our own experience, as you mentioned, was of several years ago But there are two points that I think do not fit in with the picture that you offered as a summary

In the first place, we investigated(42) the turnover rate of body fatty acids in young rats receiving choline and young rats not receiving choline, in quest, I think, of the very same thing that you are discussing, namely, that in the presence of choline in the diet, there may be more rapid oxidation of fatty acids in the liver, and we failed to find such a difference, as I recall the results

In the second place, there was an experiment(31) which was almost the counterpart of a simultaneously performed experiment or perhaps an even earlier performed experiment, in Dr Best's laboratories with Ridout(70)

In our laboratory it involved deuterium oxide In your laboratory it involved deuterium fatty acid But the results were essentially the same

We gave heavy water to animals on a complete diet and to animals on a diet deficient in choline We observed that in the animals with fatty liver there was apparently approximately normal fatty acid synthesis in the liver but definitely less than normal newly synthesized fatty acid in the depot

Artom Yes

Stetten And this we construed to mean that the effect in the choline-deficient rat was a block at some point in the transport of fat from the liver to the depot

Artom Yes, I think I tried to mention these findings and their significance

Stetten Yes, you cited those

Just as you were inclined to favor your own experiments I think all of us are inclined to favor our own experiments and I would be very anxious to know how these two sets of data can be brought into accord with your picture, as I understood your picture, that the choline deficient rat is less competent, perhaps, to dispose of fatty acids within the liver

Artom It seems to me that such a picture is today perhaps more likely, but I am very doubtful about stating that it is "the true picture" In fact, I think that many of these experiments may be interpreted in various ways Thus Dr Stetten could not find differences in the rate of disappearance of fatty acids from the tissues of rats receiving or not receiving choline But, as I mentioned before, differences in rats were small enough not to be detectable Still, a very slight decrease in the disposal of the fatty acids would probably be sufficient to account for the excess fat accumulating in the liver After all how much fat accumulates in the liver of these deficient rats? Probably not more than 1 or 2 per cent of the total fat which is metabolized or stored in a normal manner by such animals during 6 or 7 days

Another possible explanation may be suggested for the observation that after choline was given, lesser amounts of isotopic fatty acids were present in the liver and equal or greater amounts in the depots It might be that fatty acids were carried out of the liver in a form other than phospholipids, for instance, in the form of ketobodies These could then be resynthesized to fatty acids in the peripheral tissues Or it may just be that the improved metabolism of fatty acid in the liver spares some of the fatty acids already deposited in the fat tissues In other words, it is perhaps possible to give to Dr Stetten s data some other interpretations which might be reconciled with the concept that most of the action of choline is due to an increased utilization of fatty acids in the liver The data are what they are but they do not say that blood phospholipids are the carriers of the fatty acids from the liver to the depots

Stetten Oh, no, they don t say that But it may still be, may it not that phospholipid formation is essential for the delivery of fatty acid residue from the intracellular to the extracellular compartment?

Artom Yes, it might be that such a process is a preliminary stage of the mobilization of fatty acids in the liver But it is obviously difficult to make any statement on this point since we don t know how fatty acids are utilized in the liver or transported out of the liver

György Dr Stetten you feel that it has not been proven that there is no mobilization of fat under the influence of choline, from the liver to the periphery?

Artom He feels that there is

Stetten I rather suspect that under the influence of choline there is an enhanced mobilization of liver fat to the depot As to whether that appears as phospholipid in the circulating blood – that is another question

Gyorgy Therefore you are not convinced by the argument of Dr Artom?

Stetten I have not been convinced by the argument of Dr Artom

Artom I think that I would leave this problem as it is, that is not solved In all fairness one should say that, after all one objection made to the interpretation of Dr Stetten s results could apply

as well to Chaikoff's conclusion against the role of plasma phospholipids as carriers of fatty acids As one illustration some fatty acids could be carried out of the liver in the form of lecithins and transferred to tissues other than the liver These amounts must be small enough to be within the limits of error of their experiments However in the long run the failure of such a way of transporting fatty acids of the liver might well result in an increased fat content of the liver

Stetten It was with that in mind that I asked Dr Cayer the question earlier, and I think it would be interesting to find out even if the neutral fraction of the circulating blood increased in those subjects in which there was previously a lot of fat in the liver and who showed some response to methionine in such acute experiments

Best I don't think, Dr Stetten, that has ever been investigated carefully in experimental animals Do you remember?

Popper There is a German report(71) that after oral (actually duodenal) administration of 4 Gm choline chloride, the serum phosphatides rise significantly in normals and patients with hepatitis, whereas after intravenous administration of 4 Gm choline chloride (this is a pretty large dose), the phosphatides supposedly do not rise

Gyorgy In man?

Popper In man

Best From a physiologic point of view, I think it is a rather useless procedure to have phospholipids made in the liver and go into the blood and come back to the liver to be used I don't see why they ever leave the liver at all I think that Dr Chaikoff has got himself, got us all perhaps, a little restricted in our thoughts and I would be quite prepared to believe that there is a break there where perhaps fatty acids can leave the phospholipids and proceed on their way to tissues Of course, there is no evidence yet

Artom Yes

Best But it is a fertile field in which to work

Artom I would like to add here one more point which I did not mention in the preceding discussion, but which certainly has some bearing on the problem of the role of plasma phospholipids If one calculates the amounts of phospholipids which are actually turned over by the liver in a certain time, it seems that these

amounts are far below the figures which would be required for the transportation of the large quantities of fatty acids which are or can be metabolized or stored by a normal animal in such a period In other words, it seems likely that the transport of fatty acids as lecithin represents at most a mechanism Yet if the subsidiary mechanism is failing and the liver has to dispose of large amounts of fatty acids, one may understand that fat accumulates in the liver After all, when the traffic on the highway is very heavy secondary roads may become quite important and if they are not available, a traffic jam will develop

Best The only thing that was labeled in Chaikoff's experiment was the phosphorus

Artom Yes

Best And the phosphorus goes back to the liver, but the fatty acids don't necessarily have to go back

Artom Yes

Best They could be split off

Artom Yes

Best I don't know what you would put in their place

Stetten We know they form ketone bodies don't we? They were first discovered in diabetic dogs

Best *Yes*

Shorr So they do form ketone bodies

Best Until you get to an excessive fat content and then the liver fails

Turner It may be pertinent to this discussion that we have observed one case of fatty liver that had extremely low values of neutral fat at all levels in the centrifugate As far as I am aware, the amount of neutral fat distributed at all levels there is unique in its lowness We know nothing about what might have happened with choline It is just an isolated observation, but it stands out in our scattered data

Shorr Are there any data on the respiratory quotients in rats with fatty liver? Are they burning anything but fat?

Best The quotients of diabetic dogs with fatty livers go up I dont think that means really what it seems to mean But as the liver fails the quotient goes up

Shorr That is in the long surviving diabetic animal

Best Yes the long continued observation

Shorr How about the rats? Do we know anything about them?

Best I dont think there are any data Do you know Dr Stetten?

Stetten I dont know

Best In planning these experiments one would like to have only one limiting factor and if you lower the protein of the diet as well as the choline you have more than one limiting factor

It might be that we would learn more about the action of choline itself if we went back to the early experiments where you can have a diet perfectly adequate in protein but if you have it high in fat instead of high in carbohydrate you can get a very fatty liver which responds to the addition of choline And if you have no protein deficiency – well unless you figured it out on a caloric basis you might get in trouble there

Shorr That is where the guanidoacetic acid experiments would also be very helpful

Best Yes

Stetten I should like to ask Dr Artom if I understood him correctly that the esterification of the glyceryl portion to phosphoric acid requires essentially intact cells and oxygenation?

Artom Yes

Stetten Whereas the conjugation of the phosphate to the nitrogenous base proceeds either aerobically or anaerobically?

Artom Yes although it proceeds anaerobically less actively than in air

Stetten But it does proceed in the absence of oxygen?

Artom Yes

Stetten But the conjugation to phosphoric acid of the glyceryl whether free glyceryl or some substance combined with glyceryl requires a continuous source of high energy phosphate?

Artom I guess so

Knisely May I ask a question? A cell cannot use a single ion or molecule in the chemical reactions going on inside the cell until the ion or molecule reaches the cell Extending this idea the rates at which a chemical reaction for which a specific kind of ionic or molecular anabolite is or are necessary cannot possibly run any faster than the individual ions or molecules of the necessary anabolite are delivered to the cell in which the reaction is to take place In consequence of this we have been watching for some time to find out if there are specific physiological or chemical functions of the liver which might occur only when pure hepatic artery blood is flowing through the liver And we have been watching to see if there are others which might occur only when pure portal vein blood is passing through the liver In the case of pure artery blood this has a much higher oxygen concentration than does pure portal vein blood Also during those times when glucose is being absorbed into the portal vein s blood the portal vein blood passing into the liver will have a higher concentration of glucose than does the hepatic artery blood at that same time

Obviously there are other individual substances which occur in these two types of blood in quite different concentrations under given individual sets of physiological conditions In any such case the cells of the liver may be limited in their rates of supply of a necessary anabolite when the vasomotor system shuts off one set of vessels which leads a specific kind of blood into the liver

Artom You mean trying to cut off one of the two supplies?

Knisely Is there a possibility here that the control of which kind of blood is going through determines by limitation which kinds of chemical reactions can go on or the rates at which reactions can proceed?

Artom I don t know

Knisely Might this be worth investigating?

Artom It seems to me that it would be a somewhat difficult experiment not to make but to interpret Can you cut off the supply let s say from the hepatic artery and leave intact the supply from the portal vein?

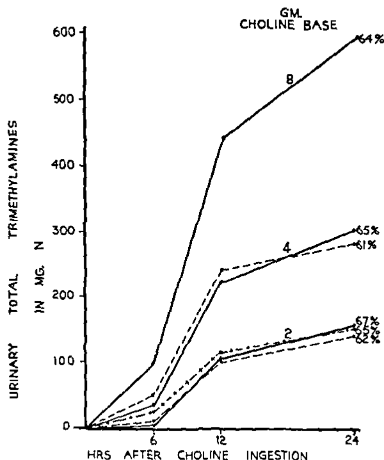
Knisely Dr Artom the liver does this the vasomotor system of the liver will simply shut off the hepatic artery at certain times

so that nothing but pure portal vein blood comes through the liver and at other times it shuts off the portal vein and only pure hepatic artery blood passes through the liver The liver may have its total blood supply through the hepatic artery, or through the portal vein (cf Soskin *et al*(72) also Knisely *et al*(73)

Best When the hepatic artery is tied (as you know Dr Markowitz reported it here) you must give antibiotics to save the animal's life at least for the time you are doing the experiment(74)

Dr Popper has some experiments where the antibiotics are involved in the action of the lipotropic factors I will ask him to say a few words about them now

Popper To start at the beginning Dr Huerga in our laboratory (75) was interested in the urinary excretion of choline after its oral administration We were hoping to obtain some information about hepatic function assuming that possibly the choline excretion after oral administration of a test dose would be increased in hepatic cell damage and decreased in biliary obstruction It turned out that in normal persons the bulk of orally administered choline was excreted in the urine as trimethylamine actually 95 per cent of it as trimethylamine oxide Whether 2 4 or 8 Gm of choline base was given 62 to 67 per cent of the administered dose (as expressed in N) appeared in the urine as trimethylamines (Figure 25) a substance which has no lipotropic activity We were wondering where these trimethylamines may come from There are reports in the literature(76 77) that intestinal bacteria especially of the Proteus and Salmonella groups but not Escherichia coli are able to transform choline into trimethylamine Therefore Dr Huerga considered a previously suggested possibility that the trimethylamines may result from a transformation of orally administered choline by intestinal bacteria Several observations confirmed this assumption It has been reported that glucose inhibits in cultures the conversion of choline to trimethylamines by bacteria(77) Therefore a person who excreted 65 per cent (expressed in N) of 4 Gm choline base administered orally as trimethylamines received choline together with cane sugar (Figure 26) This delayed the trimethylamine excretion slightly If the choline was given together with starch (not as readily absorbed in the intestine) the trimethylamines excretion was reduced to 6 3 per cent of the dose of choline given Similarly after three days of treatment with antimicrobial substances (aureomycin and sulphthalidine) only 11 4 per cent of the choline administered was excreted as trimethylamines These find



F [illegible]

ings were duplicated in a series of additional experiments even with administration of 10 Gm of choline We believe these findings indicate that in the normal human being 65 per cent (well reproducible in the same person) of choline administered is transformed by intestinal bacteria into trimethylamines whereas the rest is probably absorbed After antimicrobial treatment the destruction of choline appears very much reduced and probably a much greater

amount is absorbed Drs Meitus and Schaffner in our laboratory are, at present, trying to prove increased absorption of choline after administration of aureomycin using the P^{32} turnover procedures which Drs Artom and Cayer have just demonstrated The results so far available support these conclusions

We also tried to find out whether intravenous administration of choline which thus bypasses the intestinal bacteria, leads to a significant rise of the trimethylamine excretion(78) We were careful at first not to give too high doses of choline intravenously and started out with 1 Gm and are now giving 2 Gm without encountering difficulties The German paper previously quoted(71) even gives the impression that in Germany choline is usually given by the intravenous route Anyway, our group had given intravenously 1 or 2 Gm of choline base on over 80 occasions without noting any untoward results except for some blushing

Best That is given slowly?

Popper We gave 1 Gm in 1 hour and 2 Gm in 2 hours

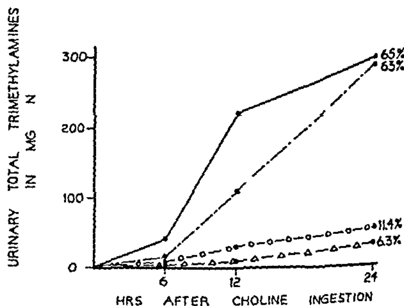


FIGURE 26 Urinary excretion of trimethylamines after oral administration of 4 Grams of choline base to a normal person without supplement (——) after four days treatment with aureomycin and sulfathalidine (—o—o—) with simultaneous administration of 100 Grams cane sugar (—·—·—) and 100 Grams of starch (—Δ—Δ—) Reprinted from Huerga J de la and Popper H Urinary excretion of choline metabolites following choline administration in normals and patients with hepatobiliary diseases *J Clin Investigation 30 463 (1951)*

In contrast to the oral administration of choline which was followed by marked urinary trimethylamine excretion and little, if any, choline excretion, after intravenous administration of choline, resulted in an excretion of approximately 10 per cent of the given dose as choline in normals. The trimethylamines excretion as measured per hour, increased slightly which may possibly be an effect of the liver in transforming choline to trimethylamines. The marked trimethylamines excretion noted on oral choline administration, however, was not noted (Figure 27). These findings again support the contention that the urinary trimethylamines originate mainly from action in the intestine and that only little is formed by other organs.

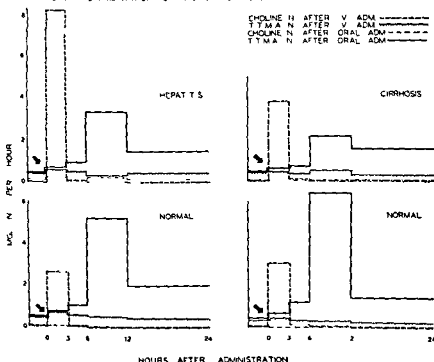


FIGURE 27. Urinary excretion of choline and total trimethylamines following oral and *intravenous administration of 1.15 Gm. choline chloride to two control patients* (suffering from fractures), one patient with acute infectious hepatitis and one patient with cirrhosis with jaundice. (A figure utilizing the same material but not identical in arrangement will appear in Huerga, J. de la, Popper, H. and Steigmann, F.: Urinary excretion of choline and trimethylamines after intravenous administration of choline in liver diseases — which has been accepted for publication in the Journal of Laboratory and Clinical Medicine.)

isolated observation in the human This supports the assumption of increased choline absorption in such rats with low trimethylamine excretion and, per analogy, also in the human with liver diseases To establish whether the increased choline absorption in liver disease might possibly be an expression of a choline deficiency, a series of patients with liver disease and low trimethylamine excretion were treated with 5 5 Gm choline base daily for 2 weeks Subsequently, the trimethylamine excretion after administration of the same standard dose became normal (Figure 28), although the liver function as measured by various tests did not improve during these two weeks *This observation would at first sight support the* contention of a choline deficiency at the time of the first test dose administration, however another explanation also occurred to us As mentioned, the literature indicates (76, 77) and Dr Huerga has supported it by extensive study, that bacterium coli does not transform choline to trimethylamine whereas other enteric bacteria do We were wondering whether or not prolonged treatment with choline might increase in these patients with liver diseases the choline transforming bacteria at the expense of non choline transforming bacteria and whether therefore the increased trimethylamine formation after prolonged treatment may not be the result of *an altered bacterial flora*

Surprisingly enough, Dr Felsenfeld found in the stools of the first six patients with cirrhosis he examined after the prolonged choline treatment a marked drop or in some cases almost disappearance of the coliform bacilli Subsequently, three more cases were studied in one of which this phenomenon of the marked reduction of the coliform bacilli from the stools did not occur Therefore in eight out of nine cases a significant change of the bacterial flora had resulted from the choline administration which could explain the altere ... tilization after ... e treatment I might stress that we ... se, uncertai ... at degree the stool really reflects ... *l flora in the* ... *ract*

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rather than an increased absorption evidence given by your earlier observations that the intravenous injection in the liver injured patients was associated with a much higher excretion? If there were a deficiency existing at that time one might not have anticipated that higher excretion Or conversely on oral administration if choline were more rapidly absorbed in a choline deficient liver injured patient one might have gotten a rise in choline in the urine just as you did on intravenous administration

Popper We feel that we may be able to answer this question only by experiments on rats with a rigid choline deficient diet which we so far have not carried out We wonder whether rapid relief of the choline deficiency would alter the trimethylamine excretion We have found reduced trimethylamine excretion in rats maintained on high fat low protein diets but we prefer to be careful in interpreting these findings Your argument would hold had we not failed to find in cirrhosis a high choline excretion on intravenous choline administration a fact which confuses us very much In contrast to acute hepatitis in which we usually found an elevated urinary choline excretion on intravenous choline administration we obtained variable results in cirrhosis occasionally we even found very low values and the average was almost below normal (Figure 30) Although in renal failure the intravenous choline excretion is low this factor does not apply in most of the cirrhosis cases To study the influence of a possible choline deficiency patients with cirrhosis received oral treatment with choline for two weeks Subsequently repetition of the intravenous choline administration did not produce consistent results Frankly I have no explanation at this time for the peculiar increase of the urinary choline excretion in acute liver disease and I want to emphasize that in describing this phenomenon

Shorr Actually Dr Popper isn't it quantitatively rather small something like from 2 to 8 mg of the nitrogen in terms of the total choline administered?

Popper The average amount of choline excreted in the urine of normal persons after intravenous administration is approximately 10 per cent of the dose given

Shorr I see

Popper This percentage may rise in acute hepatitis but not in all cases to something like 20 to 25 per cent of the dose given I would like to stress one point the normal person loses about 10

isolated observation in the human This supports the assumption of increased choline absorption in such rats with low trimethylamine excretion and per analogy also in the human with liver diseases To establish whether the increased choline absorption in liver disease might possibly be an expression of a choline deficiency a series of patients with liver disease and low trimethylamine excretion were treated with 5 5 Gm choline base daily for 2 weeks Subsequently the trimethylamine excretion after administration of the same standard dose became normal (Figure 28) although the liver function as measured by various tests did not improve during these two weeks This observation would at first sight support the contention of a choline deficiency at the time of the first test dose administration however another explanation also occurred to us. As mentioned the literature indicates (76 77) and Dr Huerga has supported it by extensive study that bacterium coli does not transform choline to trimethylamine whereas other enteric bacteria do We were wondering whether or not prolonged treatment with choline might increase in these patients with liver diseases the choline transforming bacteria at the expense of non choline transforming bacteria and whether therefore the increased trimethylamine formation after prolonged treatment may not be the result of an altered bacterial flora

Surprisingly enough Dr Felsenfeld found in the stools of the first six patients with cirrhosis he examined after the prolonged choline treatment a marked drop or in some cases almost disappearance of the coliform bacilli Subsequently three more cases were studied in one of which this phenomenon of the marked reduction of the coliform bacilli from the stools did not occur Therefore in eight out of nine cases a significant change of the bacterial flora had resulted from the choline administration which could explain the altered choline utilization after choline treatment I might stress that we are of course uncertain as to what degree the stool really reflects the bacterial flora in the intestinal tract

In closing we feel that one point especially deserves clarification namely whether the observed reduced trimethylamine formation and the assumed increased choline absorption in liver disease is an expression of choline deficiency in the sense that more choline is absorbed and less left to the bacteria or whether the observation reflects a primary change in the intestinal flora in liver disease

Madden Might that not be evidence in favor of your second explanation that it is a change in the choline utilizing organisms

different levels of the intestinal tract The content of the duodenum failed to transform choline to trimethylamines Similar observations were made in rats However in both humans and rats high up in the jejunum choline transformation starts Possibly bacterium coli which does not transform choline is found in lower levels of the intestinal tract than B Proteus

Best I think we should ask Dr Gyorgy to say something about the antibiotics

Gyorgy Dr Popper mentioned the possible relation of the intestinal flora to choline metabolism I should like to show two lantern slides pertaining to experiments on rats receiving a diet free of choline — low in protein (casein) high in fat with sugar vitamins and salts

(Table XIII) The first group received — there were 10 rats in each group — the control basal diet for three weeks and had a total average liver fat of 25 4 per cent In the second group of rats aureomycin was added to the diet with food intake controlled Statistically there was no significant difference in food intake between the first and second group The liver fat was reduced from 25 4 per cent in the first group to 12 2 per cent in the second group receiving supplement of aureomycin In the third group receiving terramycin there was even a more marked reduction to 8 4 per cent Food intake was again controlled

Animals in the other three groups received supplements of methionine to the basal diet There again the lipotropic effect of methionine was distinctly potentiated by aureomycin as well as by terramycin (Table XIII)

In the next experiment a low protein (casein) high fat diet was fed to rats for a prolonged period of time (100 days) In whole groups severe cirrhosis ascites and renal changes (nephrosis) were a regular occurrence In groups receiving supplements of aureomycin with controlled feeding *no* cirrhosis ascites or renal changes were observed

In the above experiments we observed lipotropic and anticirrhotic effect in rats receiving a low protein diet free from choline This cannot be explained by the effect of intestinal bacteria on choline It is more plausible to assume that aureomycin suppressed bacteria in the intestine which otherwise would utilize and destroy methionine Thus more methionine became available

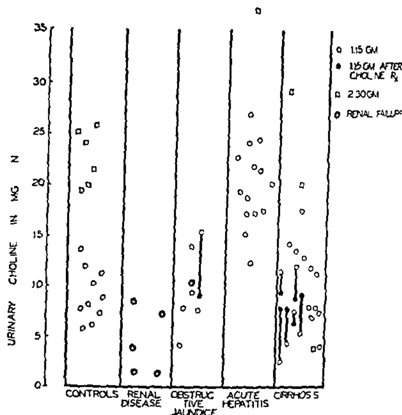


FIGURE 30 Urinary excretion of choline during the first three (four hours if 2.30 Grams choline chloride had been given) hours after the start of an intravenous injection of choline The lines connect the values obtained before and after daily treatment with 5 5 Grams of choline for two weeks (Almost the identical picture will appear in the article Huerga J de la Popper H and Steigmann F Urinary excretion of choline and trimethylamines after intravenous administration of choline in liver diseases which has been accepted for publication in the Journal of Laboratory and Clinical Medicine)

per cent of intravenously administered choline through the urine whereas 65 per cent of orally administered choline is lost in the intestinal tract

Turner Do you have any cultures of duodenal contents?

Popper Yes We studied that in the human as well as in the rat Dr Gyorgy had asked us how we could explain bacterial destruction of choline in the intestinal tract in view of the fact that the upper part of the intestinal tract where choline absorption should take place is considered to be sterile To answer this argument Dr Steigmann aspirated for us in three humans the content of

TABLE XIII

Group	Number of Rats	SEX	Diet	Treatment Per Day	LIVER		RAT WEIGHT		Food Intake GM Per Day
					Weight GM	% Total Fat	Weight GM Av at Start	% Change	
a	10	m	V_4*	—	7 3 ± 0 4	25 4 ± 2 0	155 (148-165)	− 9 0 ± 1 6	6 5 ± 0 2
b	10	m	V_4	25 mg Aureomycin	5 6 ± 0 4	12 2 ± 2 2	154 (146-166)	− 5 8 ± 3 2	6 1 ± 0 2
c	10	m	V_4	25 mg Terramycin	5 6 ± 0 3	8 8 ± 0 7	155 (145 165)	− 5 7 ± 2 8	6 7 ± 0 2
d	10	m	V_4	{25 mg Aureomycin} {15 mg Methionine}	5 9 ± 0 2	7 5 ± 0 7	152 (146 165)	+ 0 7 ± 1 9	6 6 ± 0 2
e	10	m	V_4	{25 mg Terramycin} {15 mg Methionine}	6 0 ± 0 3	7 6 ± 0 9	154 (145 162)	− 0 2 ± 2 8	6 0 ± 0 2
f	9	m	V_4	15 mg Methionine	6 2 ± 0 3	13 2 ± 2 4	154 (144 164)	− 0 8 ± 2 3	6 0 ± 0 3
g	10	m	Stock	—	8 2 ± 0 4	5 2 ± 0 2	155 (147 167)	+35 0 ± 1 2	15 2 ± 0 05
h	10	m	Stock	25 mg Aureomycin	7 6 ± 0 4	6 4 ± 0 3	155 (149 163)	+38 5 ± 2 0	15 0 ± 0 1

* V_4 = low protein high fat diet
Groups b c d e f pair fed with Group a group h pair fed with Group g

TABLE XIV

Prevention of Experimental Dietary Cirrhosis by Aureomycin

Group	Supplement	Number of Animals	Cirrhosis
A	—	10	9
B	Aureomycin	10	—

for the body and for the protection of the liver or in other words aureomycin spared methionine

Shorr May I ask Dr Gyorgy whether or not anything is known of the bacterial flora of the liver?

I think the group should know of some experiments of Fine in Boston on hemorrhagic shock in which pretreatment of the animal with oral aureomycin profoundly reduced the incidence of irreversible shock by procedures which otherwise uniformly produced this state(79) This was related to the evidence that Dr Markowitz has presented here that there is in the dog liver a considerable bacterial flora particularly of Clostridium welchii During the liver hypoxia of the shock state these anaerobes flourished and by the toxin produced as well as the damage they induced in the liver contributed to the fatal outcome The aureomycin in the gut apparently protected the animal by keeping down the growth of these organisms in the liver Inasmuch as many of us are concerned with shock in the rat I wonder whether there is any evidence for such a bacterial population in the rat liver?

Gyorgy The liver of the rat was found to be free of bacteria In normal rats and in rats with necrosis or cirrhosis of the liver no bacteria were found

Shorr Very glad to have that

Gyorgy And as far as I know in humans even in patients with cirrhosis no bacteria have ever been found Therefore dogs are a class of animals apart

Popper Our group has so far 25 negative anaerobic and aerobic cultures from human biopsy liver specimens

Sherlock Have you tried looking for viruses?

Gyorgy Well I did not but if I am not mistaken Himsworth did it in cooperation with McCallum and no demonstrable virus has been found which does not mean that there is no virus

Sherlock No but if the agent is a virus that responds to aureomycin then the virus probably is a big one

Gyorgy Well it must be big because it is questionable whether any virus responds to aureomycin with the exception of Rickettsia

Sherlock What about atypical pneumonia?

Gyorgy Do you know it s virus?

Sherlock No but it responds

Maegraith Lymphogranuloma inguinale responds very well

Gyorgy That is a large virus

Maegraith But you said no virus

Watson Don t some people question whether that is a virus?

Maegraith That is an unfair attack I think it is a definite virus

Artom Could the effect of the antibiotics be due to a sparing action on vitamin B_{12} or folic acid?

Gyorgy Yes Just as I mentioned methionine the same mechanism may apply for B 12 as well It may be that bacteria were destroyed which per se utilized or destroyed B 12 and — its lipotropic effect was protected by aureomycin

Shorr What would you estimate the methionine requirements of an abacterial animal would be?

Gyorgy That is a very good question I should like to know

Shorr Would it not be interesting to have such experiments carried out at Notre Dame?

Gyorgy With the help of Dr Watson the Liver Commission and Professor Reyneirs that will now be possible

Hanger The sterility of the liver is quite an interesting problem because you remember Opie(80) years ago injected bacteria into the portal vein and was unable to recover any of those bacteria in the liver On the other hand if you add barium salts to the liver those bacteria can be grown the answer being that the soaps of these fatty acids are highly bactericidal so bacterial sterility of the

liver is not very significant unless the action of bactericidal soaps is eliminated

Artom In relation to what Dr Popper was reporting, in our experiments with liver slices we have tried to follow some of the metabolic pathways of the isotopic choline(81) I did not mention these experiments, because I was somewhat reluctant to add one more slide

TABLE XV*

Distribution of C^{14} in Liver Slices Incubated with Methyl Labeled Choline†

Experiment No	4	5	7
Choline, lipid	0 9	0 6	0 8
Trimethylamine	0 2		0 1
Trimethylamine oxide	4 0		7 5
Choline, water-soluble, pptd as the enneaiodide‡			
(1) directly	8 2	5 8	3 5
(2) after mild hydrolysis§		5 8	4 1
(3) after strong hydrolysis‖		60 2	56 5

† The values are expressed as per cent of the counts introduced per gram of moist tissue (From Artom C and Crowder M *Arch Biochem*, 29, 227 (1950)

‡ Carrier choline was added and precipitated with I KI The values may include some related substances such as betaine aldehyde and betaine

§ One hr at 100° in aqueous 1 N HCl

‖ Refluxed 3 hr in methanolic 3 N HCl

* Reprinted by permission Artom C and Crowder M *Arch Biochem* 29, 227 (1950)

Best Everybody has a slide up his sleeve

Artom I didn't show it But now I think that some of the results may be pertinent to what Dr Popper was saying As shown in Table XV, the liver slices produced some trimethylamine and especially much trimethylamine oxide from the methyl-labeled choline Of course if there were many bacteria in these slices, they could have been responsible for this production Personally, I am inclined to ascribe this production to the tissue rather than to contaminating bacteria, but I admit that the question is not solved

Most of the choline which disappeared during the experiments seemed to be used in the formation of compounds from which

choline would again be recovered by a strong hydrolysis However, these compounds are resistant to a mild hydrolysis We at first thought of a formation of phosphorylcholine, but subsequent evidence is not in favor of this hypothesis Thus, we do not really know what these compounds are, except that they seem to be a major metabolic pathway of choline in the conditions of our experiments Of course, these are quite artificial conditions

Best Any other comments?

Sborov Dr Best, I should like to say that, contrary to what Dr Gyorgy has demonstrated as to the lipotropic effects of aureomycin and terramycin in his animal experiments, in human patients we have demonstrated that approximately one half have developed fatty livers while on aureomycin and terramycin therapy I believe this has been demonstrated elsewhere as well, by biopsies taken before therapy and after therapy In one patient where the biopsy was taken before immediately after and then following withdrawal of therapy 6 months later, the fat had completely disappeared It seemed that the fat was brought to the liver or resulted from the administration of these antibiotics

Best Would there be a great dosage difference? We have been working on a different species, but perhaps different dosages

Gyorgy He was working on patients

Sborov Patients with liver disease

Smetana I can confirm Dr Sborov's observations — in experimental animals There is moderate distribution of cytoplasmic fat in form of fine droplets in the cells about the efferent veins in animals treated with aureomycin

Gyorgy Please add that those animals received aureomycin intraperitoneally

Smetana Some of them

Gyorgy I think all

Smetana I dont know

Gyorgy I do

Best I think they would be more likely to receive a toxic dose when the material was given intraperitonally

György Surely, they not only received a toxic dose, but peri hepatitis and adhesions developed

The experiments have now been repeated in Dr Sborov's department normal animals have been given aureomycin and nothing has been found either histologically or clinically

Watson Aureomycin by mouth or intravenously?

Sborov By mouth

Shorr Was any effort made to provide for a possible vitamin insufficiency?

Sborov All of these patients received vitamin supplements

Shorr By mouth?

Sborov By mouth

Shorr Not in any other way?

Sborov No

Sherlock Did the patients have diarrhea?

Sborov Some had loose bowel movements but not diarrhea

Sherlock Prolonged diarrhea is associated with fatty change in the liver However the loose motions in Dr Sborov's patients was probably not sufficiently severe or prolonged to be the cause of the fatty livers

Sborov Loose bowel movements were not a persistent problem

Hanger Were the patients benefited by aureomycin?

Sborov We felt there was definite clinical improvement in about 25 per cent of cases Some showed increased BSP retention Some patients who had a normal BSP test prior to therapy exhibited an increased retention while on the antibiotic When the drug was withdrawn the BSP test returned to normal This appears therefore to be a temporary effect which we believe from the clinical point of view was not particularly harmful

Gyorgy There was also an increase in body weight in most of them

Sborov Some increased in body weight some lost The fatty livers were the ones that usually increased their body weight

Artom Dr Gyorgy do you have any experiments in which you gave sulfasuxidine or some other sulfa drugs which are poorly absorbed from the intestine? Did they cause the same effect?

Gyorgy I did not use sulfa drugs except in the necrosis experiment Here sulfaguanidine had a mild protective effect

Artom You mean you feel that aureomycin or terramycin are more effective?

Gyorgy Aureomycin terramycin *insoluble penicillin (given by mouth)* neomycin sulfaguanidine have an effect in that decreasing order

Best How much methionine did you have in your diet?

Gyorgy In the basal diet I had 10 per cent casein and then when the methionine was added in the last three groups it was 15 mg

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SECTION III

EXPERIMENTAL ISCHEMIA OF THE LIVER AND HEPATIC COMA

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Best I am going to read the article that Dr Rappaport prepared we regret that he could not be present at this meeting

The idea of excluding an organ from the circulation derives from the physiological experimental method of removing an organ to study its functional role in a complex organism

In the case of the liver the early workers thought that by diverting the portal blood flow to the vena cava they practically exclude the liver from the circulatory interrelationship with other organs For this experimental purpose Stolnikow first used the Eck fistula in fact devised for the surgical treatment of ascites

Hahn Maasen Nencki and Pawlow(1) were the first to combine the Eck fistula with the ligation of the common hepatic artery Their dogs survived the operation 12 to 15 hours and only sometimes 48 hours They died with wet necrosis of the liver This necrosis was mainly due to the ligation of the hepatic artery which was known to produce regularly gaseous gangrene of the liver

In 1948 experiments in the Department of Physiology University of Toronto(2) showed that death after ligation of the hepatic artery can be prevented by treating the animal with penicillin for 8 to 10 days postoperatively These findings made it possible to experiment on a more complete exclusion of the liver from the general circulation

I EXPERIMENTAL ISCHEMIA

Our aim in experimental ischemia of dogs livers was to reduce the hepatic blood supply to a minimum and to study the effects of this reduction After many trials we managed to tie off all the vessels running through the hepatoduodenal ligament into the liver

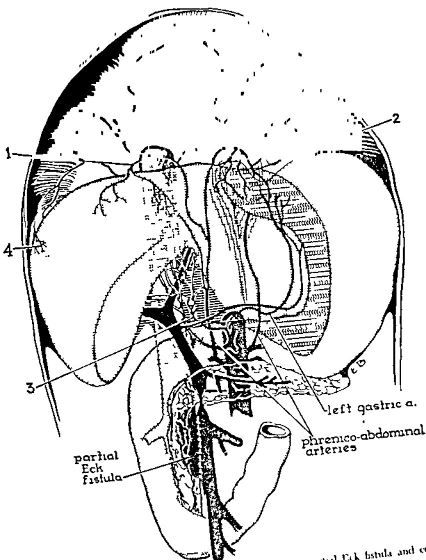


FIGURE 1 Collateral circulation of the dog's liver with partial Eck fistula and complete ligation of the hepatic artery

(1) A crown of collateral vessels around the vena cava coming up from the phrenico-abdominal arteries and sending fair sized twigs to the right and central liver lobes

(2) Collateral arterial twigs to the left liver lobe arising from the left gastric artery and its anastomosis with branches of the phrenico-abdominal arteries

(3) A plexus of collateral vessels spun around the bile duct and situated in the subserosa of the hepato-duodenal ligament sends twigs into the liver. This plexus is formed by the anastomosis of branches from the left gastric with ones from the phrenico-abdominal arteries

(4) Collateral arterial twigs coming up from the right phrenico-abdominal artery and coursing through the right coronary ligament into the liver

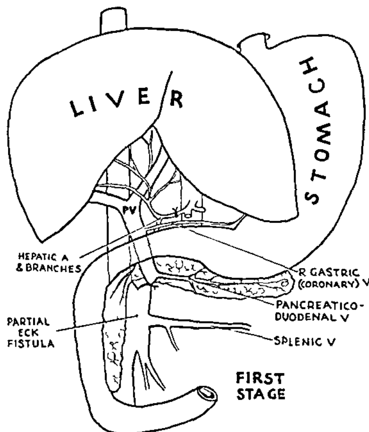


FIGURE 2 *First Stage* The stem of the common hepatic artery is ligated proximally to the gastro-duodenal artery Porto-caval anastomosis and ligation of the portal vein caudally to the pancreatico-duodenal vein (partial Eck fistula) The blood from this latter vein passes freely into the liver

We deprived the liver of all its named vessels, so that it was supplied only by a collateral circulation

We make the organ live, if I may say so, on what it borrows from its neighbors (Figure 1)

Hartroft In Figure 1 Dr Rappaport has illustrated the manner in which collateral circulation develops following ligation of the hepatic artery and creation of a partial Eck fistula Small branches of the left gastric and phrenico-abdominal arteries (Nos 1 and 2, Figure 1) have enlarged Additional collateral channels have also

developed from these arteries and enter the liver *via* the coronary and hepato duodenal ligaments (Nos 3 and 4 Figure 1)

Best The ligation of all the branches of the hepatic artery and the diversion of the portal blood totally or partially into the vena cava is fatal The ligation of the common hepatic artery combined with a regular Eck fistula in a one stage procedure is lethal too in spite of intensive postoperative treatment with antibiotics The livers showed widespread necrosis of the lytic type involving all the lobules but for small haloes of periportal parenchymal tissue

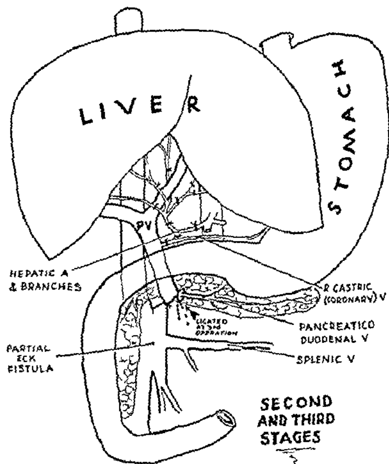


FIGURE 3 *Second Stage* All branches of the hepatic artery and the right (*coronary*) gastric vein are ligated The liver is supplied with blood (besides that from its arterial collaterals) mainly by the pancreatico-duodenal vein
Third Stage The pancreatico-duodenal vein is ligated The hepatic artery and the portal vein are completely tied off

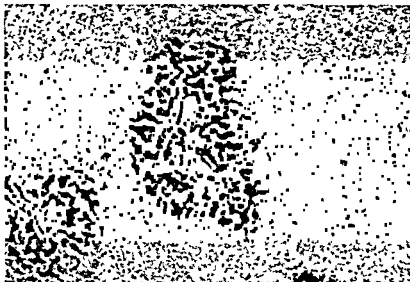


FIGURE 4 *Dog A39* Little islands of periportal tissue are surrounded by the "embracing wave of widespread central necrosis Paraffin section, Gram's stain x 80



FIGURE 5 *Dog A67* Fatal liver coma Widespread central hemorrhagic necrosis with dissolution of cell cords Varying degrees of fatty change in the necrotic cells and in the persisting periportal parenchyma Frozen section Wilson's stain Oil Red O haematoxylin light green x 20

(Figure 4) Out of 15 dogs treated in this manner only one "Blacky" a 12 kg coarse haired terrier recovered after 6 days of typical liver coma We shall talk about this dog later After many trials I finally devised a three stage procedure which was successful in keeping the animals alive in spite of greatly restricted blood flow

The first stage consisted of ligating the common hepatic artery and making a portocaval anastomosis tying the portal vein caudally to its pancreaticoduodenal tributary ("partial Eck fistula") Figure 2

In about two months all of the arterial branches in the hepatoduodenal ligament are tied off The right gastric vein is also ligated The third stage which is done 6 to 8 weeks later consists of tying the pancreaticoduodenal vein (see Figure 3)

Stage one has the most serious effects on the dogs – 8 out of 29 died Four of these (A29 A40 A53 A67) died with hepatic coma Histologically the livers showed widespread hemorrhagic and lytic necrosis with various fatty changes in the non necrotic cells (Figure 5)

Hartroft Figure 5 illustrates a typical area in a frozen section from one of these livers In the black and white protograph fatty parenchyma appears almost black and necrotic liver tissue a slightly lighter shade of grey Nearly every portal triad is surrounded by a halo of fatty parenchyma which has resisted the necrosis present in the remaining portions of the lobules Figure 6 illustrates this feature under a higher magnification A plate of liver cells–only one cell thick–which surrounds the portal canal (grey) has resisted frank necrosis but has undergone advanced fatty change (black in photograph) The remainder of the liver parenchyma is necrotic fragments of stainable fat (black) are intermixed with the degenerated cells

Best One of those dogs which will be seen in the movie went through a seven day hepatic coma and recovered

On taking biopsies 48 hours after the 1st stage operation there was no bleeding from the liver Dr Hartroft reports widespread centrolobular necrosis present in such biopsies

The second stage is better tolerated Out of 18 dogs only 3 died (bile peritonitis) At this operation the untied branches of the hepatic artery and portal vein are seen to be markedly increased in size



Figure 6 Dog A67 Around the portal canal there is a plate of fatty degenerated non necrotic parenchymal cells Frozen section Wilson's stain Oil Red O haematoxylin light green x 200

Six months after this operation one liver showed dedifferentiated pericentral parenchyma, (Figure 7) and collapse of portal areas

Hartroft Figure 7 demonstrates the type of parenchymal involution found in the liver six months after operation The primitive nature of the pericentral cells is clearly shown These are part of the same liver cords which extend towards the upper left of the field This demonstrates that the simple cells have been derived from normal parenchyma by a process of dedifferentiation and involution

Fremont Smith These findings remind me of the type of cells described by MacNider(3) as embryonic cells or flattened cells He thought they were embryonic in character, he almost always found the same thing in the tubule of the kidney They were very resistant to further injury Are these analogous to that?

Best Dr Hartroft can answer that

Fremont Smith Your description sounded as if that were true



FIGURE 7 Dog A25 Six months after second stage Central vein filled with erythrocytes There is an abrupt pericentral atrophy and dedifferentiation of liver cells The cells contain small intracytoplasmic vacuoles Paraffin section Gomori's stain oil immersion x 1000

Hartroft These do not have the same appearance as MacNider's illustrations but it may be a different condition of the same underlying process

Fremont-Smith You remember MacNider found these cells had a nonspecific resistance to a variety of substances which would ordinarily kill the animal through liver injury

Best I was talking about that tonight with Dr Hoffbauer In the cirrhotic liver of rats you see areas of regeneration with non fatty cells even though the same drastic diet is continued

Hartroft Figure 8 affords a comparison of the appearance of a typical portal canal in one of the experimental dogs and in a control animal There are definite suggestions of involution of some of the constituents of the portal triad in the experimental dog

The lower illustration is from one of Dr Rappaport's [illegible] animals The photo[illegible] nification [illegible]



FIGURE 8. *Dog D33. Upper half.* Normal portal area as control. The radical of the portal vein is filled with red blood cells. A small bile duct is visible in the left lower corner.
Lower half. Highly "cellular" appearance of the portal region due to structural collapse. The constituent cells are small and appear close together, especially in the bile duct which has shrunk to the size of a *bile ductile*. *There is also* collapse of all vascular channels. Paraffin section. Haematoxylin and eosin. x 800.

so that it takes up no more space than the smaller branch shown above with only four nuclei.

Best. A quite frequent finding was epithelial cells with iron-containing sudanophilic masses — stigmata of former focal hemorrhagic necrosis as shown in Figure 9.

Hartroft. Prussian blue stains of such livers (Figure 9) reveal the presence of scattered focal areas of no apparently characteristic distribution consisting of shrunken parenchymal cells which contain haemosiderin. In some instances Kupffer cells are found also to contain iron pigment.

Gyorgy. Were antibiotics used all of the time?

Hartroft. Yes. Postoperatively for eight days.

Gyorgy. Penicillin?

Best. Yes, and sometimes streptomycin too.



FIGURE 9 Dog A8 A residual area of necrosis in the liver The granular debris which fills the cells reacted positively when stained with Prussian blue
Upper half Paraffin section Prussian blue x 600
Lower half Paraffin section Haematoxylin and eosin x 600

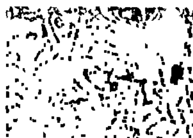
After this operation the liver is mainly supplied by the pancreaticoduodenal vein These dogs might be useful for the study of the interrelationship between pancreas and liver

In the third stage which has no mortality, the remainder of the vessels running through the hepatoduodenal ligament are completely ligated (Figure 3) The animals which have undergone this three-stage procedure are in a precarious state of health similar perhaps to a patient with chronic liver disease Some of them have bouts of so called "meat intoxication" The livers of such dogs show little change in the gross (Figure 11) Histologically there is no active necrosis There is evidence of vacuolar fat and iron loaded cells as well as an increase in recticular fibres around atrophic cells (Figure 10)

Shorr Is that iron ferritin?

Hartroft. I don t know

Γ.. *Smith* Those animals are in shock all the time?





more conspicuous due to increased reti culin fibres and early fibrosis

Lower half Paraffin section B elscl ow sky silver stain x 500
Upper half Paraffin section Connective tissue stain x 500



FIGURE 11 *Dog A9* Posterior surface of a dog s l ver 6 months after third stage R ght l ver lobes sl ghtly decreased in size Enlarged gall bladder Many adhesions.



FIGURE 9 *Dog A8* A residual area of necrosis in the liver The granular debris which fills the cells reacted positively when stained with Prussian blue
Upper half Paraffin section Prussian blue x 600
Lower half Paraffin section Haematoxylin and eosin x 600

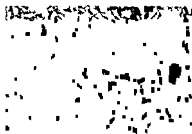
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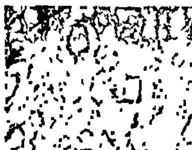
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FIGURE 11 Dog A9 Poster or surface of a dog's l ver 6 montl s after tl rd stage R ght l ver lobes slgl tly decreased n s ze Enlarged gall bladder Many adhes ons

Best No No fatty degeneration, other than a few focal areas of fat and iron loaded cells, has been observed in these ischemic livers (Figure 12)

Hartroft Cells which border the necrotic areas contain appreciable amounts of sudanophilic material (Figure 12) as well as iron pigment Such cells persist for long periods even after removal of adjacent cellular debris in the necrotic zones which they border (Figure 9) Fatty and iron laden cells of this type may be found many weeks or months after the acute episode and serve as pathological clues which indicate the nature of previous events

Best Further studies on these dogs might give valuable experimental and biochemical information

II HEPATIC COMA

The clinical manifestations of hepatic coma observed postoperatively in A24, the dog "Blacky" that survived the creation of an

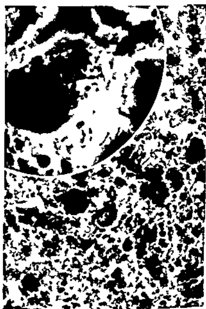


FIGURE 12 *Dog A61* Biopsy 48 hours after second stage operation At the edges of centro-lobular haemorrhagic necrotic areas there are dissociated non necrotic parenchymal cells which display sudanophilic material It contains iron and gives a positive Prussian blue reaction These lesions precede those seen in the following figure
Frozen section Wilson's stain Oil Red O haematoxylin light green × 180
Inset × 1000

Eck fistula and the ligation of the common hepatic artery in a one stage operation, made us strive for the reproduction of these symptoms at will Fourteen other trials had a lethal outcome The survival of A24 was apparently due to a well-developed right gastric vein which we observed in this dog during operation Thus its liver apparently had an additional route of blood supply besides its usual collateral arterial channels

Continuing our experiments with liver ischemia we observed a nonfatal liver coma in dog A34 after a first stage procedure which I shall show in our film record later Here a kinked duodenal loop occluded the pancreaticoduodenal vein partially but enough blood was still admitted to keep the dog alive during the critical time which the liver needs to build up its collateral circulation

We kinked duodenal loops intentionally in similarly operated dogs but without success We tried in 2 other dogs ligating the pancreaticoduodenal vein, 48 hours after the first stage operation, but the dogs recovered without any ill effects

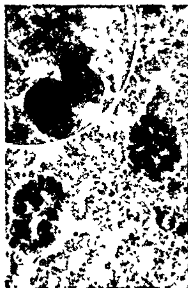


FIGURE 13 *Dog A8 Liver biopsy 8 months after second stage operation* The collections of sudanophilic material have formed from altered red blood cells These sudanophilic iron containing masses represent the stigmata of areas of haemorrhagic necrosis

Frozen section Wilson's stain Oil Red O haematoxylin light green x 180 Inset x 800

More and more the idea crystallized in our mind that nonfatal liver coma is the clinical expression of ischemic hepatic necrosis which is relieved by a developing collateral circulation Hence the success of the experiment depended upon creating an ischemia of gradual onset which can be coped with by the increasing blood flow in the expanding collateral channels(4)

In our earlier experiments we tried to use for gradual constriction a device made of Lucite placed around the vascular structures of the hepatoduodenal ligament We failed in 4 dogs because of intestinal obstruction due to this foreign body in the peritoneal cavity

Reverting to a multiple stage procedure we did a first stage operation plus ligation of the right gastric vein, and 48 hours later we tied off the pancreaticoduodenal vein The dog (A60) died and we found deep congestion and dilatation of the duodenal loop Necrosis of the liver with little fatty change was evident

The final experiment was to create an Eck fistula and ligate the common hepatic artery 48 hours later The animals (A63 A71) were treated with antibiotics and glucose infusion Glucose is given as a measure to replace a missing liver function, the production of sugar Thus we avoid the complicating factor of hypoglycemia Both dogs survived The one (A63) showed a subcomatose state for 2 days the other (A71) only for one day Three weeks after the operation the former (A63) developed ataxia, progressing to signs of a spinal cord lesion Could this spastic paraplegia of the hind legs be due to demyelination of the pyramidal tracts, such as is observed in humans with chronic hepatic insufficiency? One animal (A65) was operated upon in the same manner but not given glucose postoperatively It died in hypoglycemia, with convulsions 21½ hours after ligation of the common hepatic artery The liver showed widespread central necrosis and few fat loaded cells

To obtain a picture of a full blown coma we reduced the interval of time elapsing between the creation of an Eck fistula and the ligation of the hepatic artery to 36 hours Three dogs (A66, A69, A70) were thus operated on and treated with antibiotics and continuous glucose infusion A66 and A69 developed a typical but not lethal liver coma A70 showed no symptoms of liver insufficiency We attribute this fact to a well developed right gastric vein which we observed and did not ligate during the operation The additional blood flow through this vein might have prevented a widespread necrosis necessary to bring about liver coma

The signs displayed by the comatose dogs were Loss of appetite, vomiting, restlessness, fever, jaundice, bradycardia, stupor, somnolence, coma with deep snoring breathing muscular rigidity, convulsions and muscular twitchings ("soubresauts" of the French literature) We recorded several of these signs in a movie to be seen later Dog A66 developed neurological signs 2 weeks after it came out of hepatic coma Ataxia, lack of sense of position (the dog sometimes bearing weight on the dorsum of its feet), pronounced spasticity of the hind legs associated with muscular fibrillations, were present The animal lost its appetite completely and died a week later At autopsy we observed severe anemia, slight edema of the subcutaneous tissue and fatty degeneration of the liver, which was reduced in size Histologically there was a more advanced periportal fatty parenchymal change a similar picture as seen in Figure 6 with less central necrosis (choline deficiency?) There was condensation of reticulin in the necrotic areas of lesser degree than in Figure 10

The other dog was sacrificed 2 days after it had recovered from coma At autopsy we observed a slight edema of the ankles, the intestinal loops were adherent to the abdominal incision and the liver slightly reduced in size, showed swelling and rounding of the edges in the left lobes, they were slightly stippled with yellowish spots

Shorr Was the edema observed very frequently, or was this the only dog in which it occurred?

Best Rappaport says, "In some of our dogs with nonfatal coma, edema of the face and legs was noticed, too However, the dogs infused with 12½ per cent glucose-saline solution had good diuresis Perhaps the use of a hypertonic glucose solution has the beneficial effect of reducing cerebral edema in hepatic coma

"By inducing gradually increasing ischemia, we have produced hepatic necrosis By administering large doses of antibiotics we have decreased the bacterio-toxic factor usually present in the necrotic livers of the dogs to a minimum Thus perhaps only products of massive breakdown of liver tissue are absorbed Possibly as a result, the widespread necrosis in our experimental animals has been rendered more nearly comparable to hepatic necrosis in man

In summary, from his preliminary experiments, Dr Rappaport states that

the striate body and lenticular nucleus We wondered whether she had that type of cerebral involvement.

She was given in addition to the aureomycin 500 mg of niacin principally because of the fact that *rigidity of this type is sometimes* encountered in so called nicotinic acid encephalopathy That was emphasized by Joliffe several years ago and I think there is no doubt about it Twenty four hours after the niacin and five days after the beginning of aureomycin therapy she came out of her coma and was perfectly conscious and all of her rigidity disappeared and her Babinski reactions became negative

Fremont Smith Was the rigidity spasmodic?

Watson It was continuous

Fremont Smith Did it reach a crisis?

Watson No

Fremont Smith It was perfectly *steady all the time?*

Watson That is right steady constantly She had been out of coma for several days when I last saw her I am not sure that she is still out because these patients characteristically go back and forth — at least they often do — but she was getting along quite well when last seen

I really don t know whether it was a delayed effect of the aureomycin because we have *repeatedly seen* patients come out of coma following aureomycin and others including Dr Stokes and Dr Sborov and Dr Davidson in Boston have described apparent benefit from aureomycin either intravenously or orally

Best On what basis would that be given?

Watson Well I don t think one can rationalize too well and in fact I don t think we know why aureomycin does benefit hepatic coma if it does *We think that it may* eliminate substances coming from the bacterial flora in the colon that it may greatly reduce the activity of the bacterial flora which it *unquestionably* does and that it may in that way reduce the transmission of substances which ordinarily are handled by the normal liver without difficulty but *which are not handled by* the already damaged liver and are perhaps even permitted to go on through in the general circulation and into the brain

Best What I was really getting at was the *bacterial* growth in the intestine rather than in the liver

Watson Here is another feature, however, which may be tied up with this question of aureomycin effect, and, in fact, with the whole problem of coma, one that has interested me a great deal and one that I think offers a very real opportunity in so far as the ultimate solution of this problem is concerned, i e, the curious foetor that these patients have, the 'foetor hepaticus' This odor, as far as my experience goes, is better correlated with the appearance of coma and with the severity of the coma than any other one single sign, whether physical or laboratory We recognize that patients who have the foetor hepaticus are much more likely to develop hepatic coma Now, they may be getting along quite well, apparently, they may, in spite of outspoken signs of liver disease, be feeling well, have a fairly good appetite, and yet have this definite foetor

It has been my experience when they have it one has to be on guard as there is a very real likelihood of their slipping into coma on the least provocation Now, what provocations are particularly important? One, of course, is hemorrhage If the patient gets even a small hemorrhage from esophageal varices, he may go into coma, whereas a patient with a much larger hemorrhage from a duodenal ulcer will not become unconscious or usually will not In other words, they go into coma with a relatively small hemorrhage We have seen that repeatedly But even more trivial disturbances, such as hypodermics of morphine or demerol or a little too much barbiturate quite within the normal tolerance appears at times to precipitate coma in these individuals so that one has to be extremely careful about the patient who has this foetor hepaticus It often becomes much more severe or much more outspoken and often pervades the whole room, as coma supervenes

It isn't at all impossible to walk into a sick room and tell the moment you walk in that this patient has profound hepatic insufficiency, and if you hear stertorous breathing at the same time, you can be sure that you are dealing with a patient who has hepatic coma

We have tried on a number of occasions to find out more about this substance One of the things we have done within recent years without success is based on something I saw in Dr Best's laboratory, the use of the mass spectrometer to measure acetone on the breath We attempted over a period of two years, I believe to detect this substance on the breath with the mass spectrometer, but we have

been unable to do so It is rather difficult to know why we failed, but at least we can't detect anything

There isn't any doubt about the definition of the material. It is aromatic in character, probably very labile, it smells like an amine, it is present in the urine, one can extract it from the alkaline urine with petroleum ether, and take it back into acid, then back into petroleum ether and concentrate that down on a watch glass and note the characteristic smell So that it is a very real substance, but it is extremely hard to handle

Now, the question in my mind is, whether this substance in itself is partly responsible for coma, whether it is coming from the intestinal tract and escaping into the general circulation and damaging the central nervous system, which I think is a very real question, one that must be seriously considered We have repeatedly seen the foetor disappear or lessen promptly after a large bowel movement an enema or a short period of diarrhea, only to recur later on We have seen the foetor disappear within twenty-four hours before the patient came out of coma, which suggests that it may have something to do with it

These points are only suggestive, and of course, they don't prove anything at all It is known that amines damage the brain and produce in animals curious central nervous system abnormalities with rigidity, with catalyptic states, especially in cats deJong(8) has studied these effects of certain amines on the central nervous system

Now, the central nervous system suffers a very real injury Dr A B Baker has studied this very carefully He speaks of "hepatic encephalitis" I don't like that term very well, I don't think it is an "itis' in an inflammatory sense I think it is perhaps better designated as "hepatic encephalopathy" They do have changes they exhibit small amounts of perivascular exudate, demyelinization chromatolytic nuclei, rather nonspecific changes, although Dr Baker feels that in many instances he can say that in all likelihood the patient has had hepatic insufficiency I wouldn't be at all convinced of that from my own observations, but there are histological evidences of injury

I am extremely interested in the effect of hepatic artery ligation There is a question in my mind as to what extent one can transfer Dr Markowitz and Dr Rappaport's results to man At least what we see in man from the obstruction of the hepatic artery is usually

infarction of the liver, and so far as I have seen, there has not been any bacterial overgrowth, either with or without infarction One sees multiple infarction at times, more often in cases of periarteritis nodosa, but in certain other instances where the hepatic artery is compromised

These perhaps are not strictly comparable to the animal experiments, because in many of them, at least, the hepatic artery may not be obstructed completely and it may be obstructed more slowly, giving rise for better collaterals

I think that the question of concomitant pressure on the portal vein is important, too, in patients who have had infarcts, some of them because of tumors or inflammatory reaction also have some compromising of the portal vein which undoubtedly predisposes them to infarcts, but one rarely sees diffuse necrosis

As I say, I have not seen nor have I read about an overgrowth of anerobic bacteria in the human liver under these circumstances One certainly doesn't see the picture of gas bacillus involvement of the liver such as one sees when there is a gas bacillus septicemia in the patients, so that in that respect I think this is perhaps different Patients with multiple hepatic infarcts may in some instances live for quite a number of days without hepatic coma I think the development of hepatic coma is rather variable in patients with infarcts

Dr Hoffbauer has studied a remarkable case of multiple infarction of the liver in which there was survival for a rather long period of time If the Chairman would permit, I think it would be interesting to hear about it and see his slides

Hoffbauer As Dr Watson has intimated, nature occasionally carries out experiments in man that are analogous to those that Dr Rappaport has conducted in animals Several years ago Dr Richard Cullen and I had an opportunity to study an unfortunate man who had a carcinoma of the head of the pancreas The cancer gradually grew, obstructed the common bile duct and produced

I shall describe

cluded both the

Peritoneoscopy was performed several weeks before death This provided an opportunity to visualize the liver and to perform liver biopsies At the time of peritoneoscopy, I could not visualize the

gallbladder, although later at necropsy it was shown to be distended. The right lobe of the liver was an intense greenish gray in appearance, and exhibited numerous yellow patches I incorrectly interpreted these as metastases At that time we were taking biopsies with a needle under direct vision with a peritoneoscope in place Tonight I noted with interest that Dr Best reported the absence of bleeding from biopsy sites in the dogs Dr Rappaport studied I had the same experience in this man, namely that no bleeding occurred from the needle biopsy site I did not interpret this correctly at the time but this later proved to be from an area of hepatic necrosis rather than a metastasis One biopsy included an area of necrosis and an adjacent zone of inflammatory cells, as well as an area of relatively normal tissue

The man lived 19 days after this operative procedure, his ascites reformed He lapsed into coma approximately two weeks after the biopsy was performed and died 5 days later This was in 1946 We were not employing antibiotics in patients with liver disease at the time, this man received none of those now in current use At autopsy it could be demonstrated that the neoplasm of the head of the pancreas surrounded and compressed the portal vein and hepatic artery This resulted in a marked reduction in the lumina of these two vascular structures, but did not completely occlude them Examination of the hepatic artery and portal vein throughout their course failed to reveal any thrombi or other gross pathologic changes in the vessel walls The liver weighed 1850 grams The right lobe appeared slightly larger than normal, the surface exhibited several yellowish areas of irregular outline On cut section, these areas were a dull yellow in color, softer than normal and showed a loss of normal liver markings They were demarcated from the remainder of the liver substance by a narrow hemorrhagic zone The left lobe was smaller than normal, its surface was dark red in color On cut section this lobe was mottled with irregular soft necrotic yellow areas The needle biopsy puncture wounds were evident on both the right and left lobes and could be traced into the liver substance There was no evidence of healing at the puncture site, there was no evidence of recent bleeding at these sites

On microscopic examination, sections of the liver revealed extensive areas of infarction in the right lobe and complete necrosis of the left The signs of biliary obstruction (pigmentation and dilatation of the bile ducts) were apparent in the remaining liver tissue

We can thus document an instance in which a patient survived extensive infarction of the liver for at least 19 days This survival

could not be attributed to an effect of antibiotics since none were employed

Best Going back a minute to the complete hepatectomy these animals go into an irreversible coma always of course With complete ligation of the hepatic artery and antibiotics there is no coma whatever but Dr Rappaport has devised a method by which he can produce a fatal coma or a reversible one based of course on these three last dogs

There is of course no evidence that this is the same picture that you see clinically but it does provide an experimental procedure with production of a coma which can be studied intensively

Watson I think it is suggestive of the picture you see clinically I am inclined to think it probably is the same thing

Sborov Is there a foetor hepaticus?

Best I avoided that I find that Dr Rappaport observed pronounced foetor hepaticus in some of the dogs with advanced chronic ischemia of the livers However this classical sign was not prominent in the acutely comatose animals but this situation requires much further study

Watson That is a good question It occurred to me too

Best Are there other questions?

Popper I take it that massive necrosis with wiping out of all the cells of one lobe is not part of the picture

Hartroft In the animals which survived there was evidence that necrosis had previously occurred in scattered focal areas

Popper I also assume that wide scars due to collapse of the framework of the lobules were not noted in the animals which survived long enough

Sherlock Those areas had no reticular collapse at all

Hartroft In the areas which have been interpreted to represent regions where focal necrosis had previously occurred there is only a small amount of collapsed reticulin This is explained by the fact that material obtained from other animals which did not survive suggested that in areas involved in this type of necrosis not only was the parenchyma destroyed and eventually lysed but also the bulk of the supporting stroma

Hill Do you think in cirrhosis where you have hepatic coma the Eck fistula plays a part in short circuiting between the portal triads and the central lobular vein? I have found that very common and Moschowitz(7) mentioned it about three years ago I was wondering in the view of the possible intoxication from the gut do you think that short circuiting straight through the liver has any role to play in the production of hepatic coma?

Watson I don t know I am inclined to suspect it may have but I really don t know

Campbell I noticed the infarction in Dr Hoffbauer s case was very much more marked in the left lobe I think Dr Hoffbauer you said it was complete necrosis there as opposed to patchy on the right Is that right?

Hoffbauer I think that is correct

Campbell That is rather interesting I hesitate to mention the case I am thinking of because I can t remember what the primary operation was for I believe it was a partial gastrectomy for pyloric carcinoma The patient died about five days after operation and at autopsy there was very nearly complete infarction of the left lobe of the liver with little or none in the right lobe No thrombosis of portal vein or hepatic artery was found and the infarction was put down to persistent spasm of the hepatic artery from operative trauma One wonders if there is any significance in the left sided location of this infarction Could we postulate the synergistic effect of toxin absorbed from the large bowel coming up the left side of the portal stream? Or have we any information about the relative oxygen tensions in the two halves of the portal stream? Could that possibly condition this heavier incidence of necrosis in the left side of the liver? I don t know what the oxygen tension of the splenic venous blood is

Hoffbauer I don t know that that has ever been measured

Watson I have never heard of any data on that point at all Of course as you know Dr Himsworth suggested that it was a matter of nutrition The idea that there are substances coming from the large bowel that are much more injurious to the left lobe than the right is rather appealing to me I think that that is a distinct possibility It appeals to me more as a matter of fact than the nutritional concept especially in a case like this

Campbell But if one could explain the whole thing on an anoxic

[illegible]

rather poorer portal supply

Hartroft A factor to be considered in relation to Dr Campbell s point, is the comparative efficiency of the collateral circulation which develops to supply the right and the left lobes of the liver It is possible that the right receives more blood through such sources than the left for the right lobe is better supplied by small vessels in the coronary ligament and from the diaphragm

Popper In the last issue of *Gastroenterology* Baggenstoss and his co workers from the Mayo Clinic(8) report a very large number of infarcts in the human liver As far as I remember they do not emphasize a difference between the right and left lobe and they describe large infarcts in the right lobe The part of their study most interesting to me is that only in less than half of their cases occlusions of the hepatic artery were demonstrable and that in a third of the cases no vascular occlusion at all could be demon strated The authors discuss the possibility that hepatic infarcts may develop with or without suppression of the portal vein circula tion due to other local or systemic factors some of them disposing to anoxia They consider severe anemia shock cardiac decompensa tion as well as pressure effect from mechanical trauma Nutritional disturbances could obviously be added

We have been very much interested in correlating the histologic picture in autopsy specimens of the liver with the presence or absence of hepatic coma before death My co workers gave me a series of slides as unknowns and I was unable from the histologic picture to recognize whether the patient was in coma or even in hepatic failure or not We even failed in instances of obvious hepatic coma to notice histological evidence of necrosis At present we are apparently unable to recognize the existence of hepatic failure from the histologic picture

Drs Elias, Petty and I recently studied extensively the anasto moses between portal and hepatic vein to which Dr Hill has made reference and we found them only in cirrhosis where we ascribe them great functional significance

I would like to report at this time some experiments which Drs Koch Weser and Huerga have been carrying out in our laboratory They gave injections of bromobenzene to rats In a matter of a few hours severe convulsions occurred some of the animals survived The survivors failed to show neurologic manifestations However they and other rats receiving smaller doses of bromobenzene developed within 24 to 48 hours extensive hepatic necroses histologically somewhat similar to what we have just seen Bromobenzene is detoxified in the liver by combination with cysteine and by acetylation and excreted in the urine as mercapturic acid With depletion of cystine methionine seems to be utilized for cysteine formation resulting in a depletion of both sulfur amino acids The extensive necrosis developing within 24 hours is sometimes hemorrhagic and may then resemble histologically the massive hepatic necrosis as produced with yeast diets by Himsworth Gyorgy and others(9) The bromobenzene hepatic necrosis as well as the convulsions can be prevented by administration of stoichiometric doses of methionine or cystine Necrosis is more marked in animals which have fasted before receiving bromobenzene and histologically a good correlation exists between the duration of fasting and the extent of necrosis The association of hepatic and central nervous system lesions reminds us of Wilson s disease in which probably a similar deficiency of sulfur amino acids is caused by urinary losses(10) Apparently there are instances in which damage to the brain and liver occur simultaneously Probably in the type of hepatic coma presented here the damage to the brain is secondary to the hepatic injury

Dauphinee We are just as convinced about the importance of "foetor hepaticus" as Dr Watson is It has been compared by some of our clinicians to the smell which one can detect from pigs liver I wonder if Dr Watson has ever attempted to isolate the substance from a pig liver which is responsible for the odor?

Watson No but I am aware of that I think that is correct

Dauphinee Dr A G Gornall and Dr H Kalant(11) in our laboratory have made a few preliminary observations on the blood chemistry of some of the animals which have been used in these experiments by Dr Rappaport It has been a little difficult to dis [illegible]

those which have been actually caused by the [illegible] ligation of the hepatic artery

There is some suggestion that shortly after the second stage of the procedure there is a decrease in the level of the urea nitrogen The relative proportion of the serum protein fractions in most of the animals seems to be reasonably normal and the level of the total cholesterol is but little decreased Even the ratio between the free and the ester cholesterol does not seem to be disturbed to any significant degree and in the few determinations that have been made the percentage of free cholesterol was found to be in the neighborhood of only 30 35 per cent of the total quantity even when the animal was stuporous We have not made any observations on the serum cholesterol content of animals which have been in a truly comatose state

Shorr Were analyses made of blood uric acid?

Dauphinee No

Popper Do you have N P N determinations?

Dauphinee Non protein nitrogen determinations have been done on two dogs The N P N has been a little elevated and determinations of the various constituents of this N P N suggests that there is a larger than normal amount which is not urea and which is not alpha amino N A similar finding I think was shown by Maddock in his hepatectomized monkeys(12)

Popper In the human this is rather characteristic

Watson May I just add in that connection that it isn t uncommon in the most outspoken hepatic coma in human beings to have a rather marked elevation of urea nitrogen You see it again and again

Dauphinee A marked elevation

Watson That is right

Fremont Smith Are there any observations on the cerebro spinal fluid in terms of coloration?

Watson It is usually normal in color There are occasional cases in which it becomes yellow but they are very exceptional

Fremont Smith I had two cases with yellow spinal fluid both in acute yellow atrophy but I can t be sure about the question of coma It was noted accidentally in the first case that the color disappeared upon exposure to light When I went to the laboratory to see the fluid it was completely colorless I noticed it had been standing in the sunshine

About two years later I had another case The pigment whatever it was was not removed by any of the ordinary fat solvents we just tried adding strong acids and strong alkalis and found they had no effect at all However the pigment was very rapidly decolorized in sunlight A comparable sample kept dark maintained the pigment for a long time I have wondered often what that might be

Watson Well I can say a little bit about that It was probably not bilirubin I have not had a chance to study it I wish I could have the opportunity But I have heard others refer to the phenomenon

Gyorgy Twenty years ago we observed a green color in the spinal fluid similar to that of riboflavin

Fremont Smith And it disappeared under light?

Gyorgy Yes

Fremont Smith I believe there have been some cases of extreme jaundice where the central nervous system has been colored as if the barrier between spinal fluid and tissue had broken down but I don t know of any systematic observations on that

Sherlock I am sure it occurs wherever the jaundice is sufficiently deep It is just we don t do lumbar punctures on severely jaundiced patients I think if we did more often we would find it The pigment in the cerebrospinal fluid certainly is not straight bilirubin

Fremont Smith Is there in the blood in jaundice any substance which would be decolorized by light?

Watson Not that I know of That doesn t mean there isn t one

Turner I have had a similar experience to Dr Sherlock s study and find yellow color in many of them I did not notice the decolorization by light

Fremont Smith I may be wrong but I thought some cases of rather severe jaundice didn t have any yellow color It isn t a question of bilirubin in the blood is it?

Watson I don t think it is

Turner If it were you wouldn t expect to have something come through which would be decolorized by light

Sherlock I can recall a case of jaundice in a man with meningo ascular lues who had the deepest yellow cerebrospinal fluid I have ver seen

Turner Because the barrier was already broken down

Sherlock Yes

Schorr Were there any changes observed in the kidneys of these nimals?

Hartroft In most cases there was extreme congestion present iroughout the kidneys

Popper Were pigment casts found?

Hartroft Pigment cases were not found In some instances, oudy swelling of the tubules was noted, but other pathological ianges were not present

Shorr Were blood pressure observations made?

Neefe I don t suppose there has been any opportunity to study lectrolytes yet

Best Not yet

Dauphinee The plasma potassium content was decreased to 11 r 12 mg per cent in the blood of those dogs in which these deter iinations were done Some of these animals however, were receiv ig saline solutions and the serum sodium was apt to be elevated Vhether or not the potassium level was decreased by the sodium hloride administration or as a result of the ligation of the hepatic rtery and the Eck fistula I cannot say but there was no evidence iat the plasma potassium in any of these animals was ever ele ated above the normal range

Best It does provide an opportunity for Dr Rappaport and we ant to make a very careful study on the dogs before they are perated on, in coma and after they recover If we can manage that certainly will be done this next year

Macgraith Was there any great reduction in urinary volume?

Best I don't think there is any accurate observation on it

Maegraith Was any study made on the antidiuretic substance hat Dr Ralli has been working on?

Best No studies were made

Shorr We made observations of one patient who died in hepatic coma There was a high concentration of ferritin in the blood at death

Hartroft Were you able to demonstrate the presence of ferritin in the kidneys?

Shorr No analyses were made of the kidneys

Watson Do you think there may be a reduced sensitivity of the posterior lobe under those circumstances? Very often they dont have edema and they dont tend to go into shock It isnt uncommon for a patient in hepatic coma to remain in fairly good circulatory condition for days without edema

Shorr However if they have even a relatively small loss of blood they are very prone to go into shock

Watson Yes I think that is true but I wondered if one wouldnt expect them to get into trouble earlier with so much ferritin unless there was a reduced sensitivity

Shorr This particular patient had ascites and edema

Watson You feel do you not that the effect is mediated quite completely through the posterior lobe?

Shorr Yes through the posterior pituitary I think we have shown that very clearly

Sherlock It would be worth paying attention to the circulatory status of these animals I have been very impressed with the fact that patients in liver failure have a hyperdynamic circulatory state i e they have an increased cardiac output high pulse pressure raised venous pressure in the neck and warm extremities This would fit with Dr Shorrs observations on V D M Professor E P Sharpey Schafer of St Thomas Hospital did an experiment on one such patient He took the plasma from the patient and injected it into the brachial artery of a normal subject and measured an increased forearm blood flow in the subject You cannot draw any conclusions from that one observation but I do think it would be worth directing attention to the circulation in hepatic failure

Neefe Does anyone know the effect of shunting the hepatic artery circulation into the portal vein?

Hoffbauer It is very difficult

Popper Markowitz and Rappaport discuss this question in their recent paper in *Physiological Reviews*(13) They refer to studies(14) which indicate that hepatic necrosis after ligation of the hepatic artery can be prevented by shunting arterial blood into the portal vein

Best Yes you are right

Sborov We have done that in our laboratory We have also done liver biopsies on these animals No liver function changes have been noted

In the hepatic vein there is the same oxygen tension that one finds prior to the shunt

Knisely May I show a slide please? The slide shows the hepatic artery and portal vein running beside each other longitudinally between two liver lobules The terminal part of the hepatic artery has branches which go to the sinusoids Also the artery has larger branches which in general form loops and go over and join the sides of the accompanying portal vein These cross anastomoses from arteries to vein we have called arterio portal anastomoses [illegible] 7 9 10 and 11 on pages 28 [illegible] Warner Selective Phagocy [illegible] elements have been seen in frogs frequently and though we have looked in but a few monkeys some of them have been seen in monkeys Now to some of the functions of these arterio portal anastomoses Under some as yet unknown conditions the tips of the hepatic artery open up pouring arterial blood directly into sinusoids by branches of the artery and pouring torrents of blood through arterioportal anastomoses into the portal vein which then conducts pure arterial blood into all of the sinusoids

Sherlock In the portal tract?

Knisely Yes The pressure can raise so high it runs nothing but arterial blood through the sinusoids

You just tipped me off Dr Neefe what would happen if you did it surgically You are reproducing one of the states which the liver can go into (The liver has many physiological states)

Hill This is only in animals?

Knisely I think so far we can say we have not bothered to look in people

Hill Why?

Knisely Because of the shortness of life, we have not yet had time to look

Watson Would your quartz rod technique be feasible for this?

Knisely I think it is technically feasible, Dr Watson

Popper Dr Elias(16) showed in the human that branches of the hepatic artery extend into the lobular parenchyma and assumes that such hepatic arterioles bring blood to the central part of the lobules, other arterioles empty into the peripheral part of the lobule in keeping with the studies of Dr Knisely

Madden If it didn't, where would the hepatic artery blood go?

Popper A part of the blood goes into the portal triads The hepatic artery supplies the capillaries of the portal triads and especially the dense capillary network around the larger and smaller bile ducts, from where it enters the sinusoidal system I do not think Dr Elias's injection preparations can decide how much of the blood passes directly into the sinusoidal system of the lobules or how much goes first through the capillaries of the portal triads but following Dr Knisely's observation, there is probably a constant change depending upon the needs of the organism

Hill The arteriole goes into the lobule We see it in our cases because they have a collagen sheath

Moschowitz(7) described a similar thing three years ago His explanation is that it is part of the granulation tissue with all the formative tissues around it *I believe it is something that* is already there I think there is quite a lot of evidence showing that this point of view is right

Popper I believe Dr Moschcowitz assumes portal vein hepatic vein anastomoses and not hepatic arterial-hepatic vein anastomoses

Hill If you look at his photographs he also shows very close together what I would call two portal triads right in the lobule and he says it is due to granulation tissues growing into the lobule I don't think so myself

Popper I would agree with you

Knisely Almost every sinusoid gets a side branch from the hepatic artery That is true in frogs and also in rhesus monkeys, it *is* a part of the standard healthy anatomy and physiology

Shorr If you tie off every vessel but the hepatic artery in the rat, the liver apparently still receives enough blood to function normally There is no depression in oxygen consumption to judge from *in vitro* studies of slices from such livers One may conclude that the hepatic artery alone adequately nourishes the parenchyma

Best *I think we must adjourn at this point*

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SECTION IV

MICRO-ANATOMY OF THE HEPATIC VASCULAR SYSTEM

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I WANT TO TALK about some aspects of the anatomic and dynamic make-up of the liver circulation

In what I say there may be differences in both observation and interpretation with other workers in the same field This should not alarm you as it is always very difficult to repeat biological experiments, especially as exact details of experimental conditions are so seldom given in published work

We have formed our own views of the anatomical vascular arrangements in the liver, details of which are given in the presentation I intend to lay on the table as read So far as the dynamic attributes of the liver circulation are concerned, we believe we have evidence that in some species of animals the constrictor properties of the hepatic venous tree are significant factors in the control of the hepatic circulation and we suggest that hepatic venous constriction may be intimately concerned in the control of the blood flow through the organ and in the pathogenesis of lesions which appear in various disease states including malaria and blackwater fever

Other observers may not agree with our interpretations, but differences will clear up with time We are, however anxious to emphasize the great importance of regarding the vascular system of an organ such as the liver as an integral and specific part of the organ and not just part of a consistently constructed and separate circulatory system

We believe that attention to the specific individual properties of the liver circulation will lead to a much better understanding of the pathogenesis of lesions in that organ(1)

HEPATIC VEIN IN ANAPHYLACTIC SHOCK

Weil(2) showed that in dogs the smaller tributaries of the hepatic vein are able to constrict when he injected small quantities of

antigen locally into the liver of anesthetized dogs he obtained a local response only We have confirmed this and have extended these observations to rats and guinea pigs(3) Larger quantities give rise to more widespread congestion presumably by giving rise to constriction in larger tributaries

REACTIONS TO ADRENALIN AND ACETYLCHOLINE OF THE PERFUSED DOG S LIVER (ANDREWS ET AL(4))

Technique Livers have been perfused with Ringer Locke solution whole blood and blood diluted (up to 50 per cent) with Ringer Locke The operation of isolating the liver was performed in stages and throughout the operative procedure the organ received oxygenated blood Whilst the portal vein was being cannulated the hepatic artery was left intact. On completion of this stage the liver was supplied with blood oxygenated by a Hooker oxygenator via the portal vein whilst the hepatic artery was in its turn cannulated The outflow was taken from the inferior vena cava firstly from the abdomen and secondly from the thorax By this means the hepatic circulation was never completely stopped

Results It appears that the liver can exist in one of two states which we have called "blue" and red In the red state the color is normal there is no swelling and with the arterial pressure at 120 mm Hg the blood flow is approximately a quarter of the calculated output of the heart

In the blue state the organ is congested purplish frequently mottled and the blood flow is greatly diminished

The artery supplies a larger amount of blood relative to the portal vein when the liver is in the blue state The red state exists at the start of a successful experiment and later changes to the blue this change is hastened by stopping the circulation for a few minutes (anoxic anoxia) and the use of defibrinated blood as opposed to heparinized blood

The blue state has proved reversible on two occasions the liver regaining the color and reactions of the red The blue liver may possibly be that seen in anaphylactic and other forms of shock

Adrenalin

(a) In the red state

All doses from about 1 γ upwards effect a decrease in inflow (arterial and venous) a decrease in outflow and a diminished organ volume

(b) In the blue state

Doses from 1 to 2 γ upwards effect a decrease in inflow an increase in outflow and a diminished volume Larger doses i e 10 or 20 γ upwards act in the same way but to a greater extent on the inflow and liver volume but give rise to a diminished outflow

Acetylcholine

(a) In the red state

Doses from 0 5 to 1 γ upwards effect an increase in volume and a decrease in outflow The effect of the inflow is usually negligible with small doses but a slight increase has sometimes been noticed

(b) In the blue state

Doses of up to 20 γ seldom have any effect When there is a reaction it is similar to that obtained in the red state

The difference between the red and blue state is apparently due to impedence of the outflow of blood by constriction within the hepatic venous tree If the liver in the blue state is gently squeezed on relaxation the inflow increases but the outflow drops to nearly zero until the liver is again swollen and there is sufficient pressure to overcome the resistance of the hepatic veins

Adrenalin reduces the inflow to the liver In the red state this is reflected by an overall reduction of blood flow In the blue state the reduction in the outflow is probably overshadowed by the dilator action of adrenalin on the hepatic veins unless large doses are used in which case the inflow is sufficiently reduced to affect the outflow (It is possible that the increased outflow following the injection of adrenalin in the blue liver results partly from a squeezing effect the action of the drug being constrictor on all vessels but least pronounced in the hepatic venous tree) Small doses of acetyl choline do not appear to affect the already constricted hepatic veins of the blue liver but large doses may do so

Acetylcholine has a greater effect when introduced via the hepatic artery than via the portal vein This suggests to us a different vascular pathway through the liver A similar difference between

the effects of arterial and venous injection is also obtained with adrenalin

First, may we consider some anatomical aspects The lesion that is typically seen in blackwater fever is shown in Figure 1

Hill Is that central congestion right throughout the liver or is it patchy?

Maegraith Well, it is patchy It is more in one lobule than another But you will find it everywhere

Hartroft Do the collections of red blood cells represent congestion or hemorrhage?

Maegraith Congestion

Knisely For years I have watched histo pathologists presenting slides alleged to show "congestion" And none of the slides that I have seen which were alleged to show "congestion" has contained anywhere as near as much blood in the sinusoids as is stored in sinusoids during some phases of perfectly healthy normal physiology The difference between the amount of blood found in the liver in histological sections taken when no congestion is thought to exist

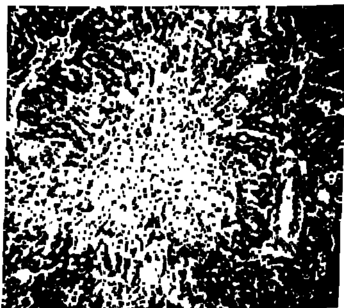


FIGURE 1 Centrilobular degeneration and necrosis in fatal case of blackwater fever

and compared to the larger amounts of blood cells found in the sinusoids and deduced to be a result of congestion" during life that difference is nowhere as great as that which exists between a) sinusoids which are constricted tightly shut throughout their lengths and contain no blood compared to b) the amount of blood cells founds in sinusoids dilated following tight closure of the hepatic outlet valves and consequent physiological filling of the liver with stored blood

Maegraith Well thank you very much Dr Knisely I should like to say that I am not a pathologist so this is congestion

Best How do you distinguish congestion from hemorrhage?

Maegraith In hemorrhage the blood is outside the vessel

Best And this is all in the vessel?

Maegraith Yes

Sherlock How do you know that the blood is all in the vessel?

Maegraith Only by observation

Sherlock On those histological sections?

Maegraith I think we are firmly limited in our views by observation and it seems by careful checking that those cells at any rate in the vast number are inside the vessel And if they are inside the vessel I think it is reasonable to call it congestion and not hemorrhage

Smetana Is the central vein preserved?

Maegraith Yes

Hill It is empty in that specimen isn t it?

Maegraith It is in that picture and the reason is that the liver was cut without the vessels being tied If you take care how you prepare your preparations beforehand you can keep the vessels and their contents intact

Sborov Are there cells in all of the sinusoids?

Maegraith I should not like to answer that I should think that there are but I could not say Perhaps it would be better to say that there is just a lot of blood in that region and leave it I don t know how it gets there

Sherlock You are not suggesting that it is a post mortem appearance?

Knisely Well, it could be

Sherlock But exactly the same appearance is seen in liver biopsy sections on patients with heart failure

Knisely Certainly autopsy sections do not necessarily record where things were before death

Maegraith Well, I couldn t agree with you more

Knisely Let s not distract you from your theme, sir

Maegraith Oh, you are not After a lot of searching just perhaps much the same as I am getting now, we came down to this general picture of the circulation through the liver (Figure 2)

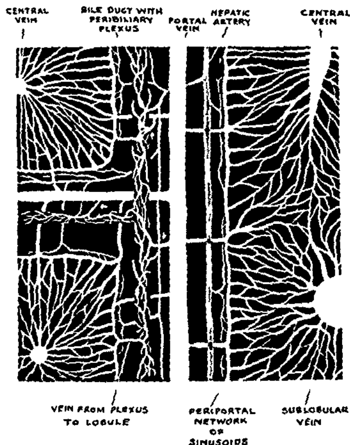
The diagram shows our conception of a portal tract with the portal vein in the center, giving off branches which run immediately to the sinusoids, and also branches which go to this remarkable plexus around the bile duct

The points of interest are that to all lobules there go both arterial and venous branches We are not in a position to say whether the arterial branches necessarily remain separate or whether there is an anastomosis there in the true sense of the word But the very strong impression we get is that the lobules do get mixed blood as a rule But, of course, this is just a dead preparation and it is not possible to say for certain what the physiological picture is in life

The thing that impressed us most is that the sinusoids on the periphery of the portal tract join up in a sort of sleeve, a continuous sleeve of large sinusoids, which, as far as we can make out run from one end of the portal tract to the other

This I think has actually been described before in the older German literature It seems to me that it is a very important part of the lobular circulation and one which may, in some way, explain why it is that so often the peripheral cells remain intact when the central ones are completely destroyed

There are lots of other points about this circulation that I might bring up For instance, whether it is an artefact or not, there is an astonishingly large number of these small branches coming from the portal vein which show a very obvious constriction at the point at which they leave the main trunk I don't know what these constrictions are, perhaps some sort of sphincter mechanism, or it may be just simply our technique



F[illegible] A medium sized portal
ranch to the left Three
ier with one sublobular
ave been omitted The
periportal network of sinusoids is shown as a continuous vessel on either side of the portal tract; its size is somewhat exaggerated in comparison with the other sinusoids The arrows point to the vessels running between the peribiliary plexus and the portal vein For the sake of clarity the number of veins passing from this plexus to the sinusoids has been reduced. Reprinted by permission from *Ann Trop Med* **43** 230 (*Plate V*) (1949)

Gyorgy Human liver?

Macgrath Yes This diagram illustrates the vascular arrangements in the pig dog rat and rabbit liver

Gyorgy No difference in principle?

Maegraith They are all basically the same, I think, sir They all have variations

Knisely Dr Maegraith, that is the position of the inlet sphincter on livers we have seen Those things open and close

Maegraith That is what we rather suspected I would not want to draw any conclusion from that, but it is a most striking fact that you find these constrictions at the point where the vessel leaves the portal vein

Knisely We don't know enough to make a definite statement

Dr Louise Warner stained some tissues from guinea pigs which had been given C vitamic acid introperitoneally In guinea pigs so treated, cells stained, at the points under discussion, like myofibrils found elsewhere(5)

Maegraith We have tried histologically to find out if there is muscle, and we cannot see it

Knisely You won't see anything very much by ordinary staining for muscle

Maegraith Figure 3 is a rather poor photograph but it illustrates very well the tremendous plexus round the bile ducts There must be an astonishing amount of blood going to it It goes right up over the gallbladder I would be very much interested to hear from you what you think the function of this particular plexus is

One interesting thing is that it not only receives blood from the [illegible] -ives out these small collect- [illegible] sinusoidal circulation We [illegible] *ls going to the* sinusoidal circulation — I don't know which way the blood goes but certainly if it is going that way it is taking something from that plexus

I think this is an interesting point that has been pretty generally overlooked

Hoffbauer I have seen similar preparations in Dr Jean Weston's laboratory in Philadelphia I recall that several years ago he wondered whether these minute bile ducts might not have some function analogous to that of the renal tubules Each structure has a rich blood supply Perhaps Dr Knisely knows whether he has made any further observations



FIGURE 3 Neoprene cast of guinea pig's liver Note peribiliary plexus and collecting vessel (radicular portal vein) joining the plexus and sinusoids

Knisely No

Macgraith You know the tremendous removal of fluid from the bile duct in diseases like blackwater fever and malaria is rather striking and does suggest that there could be a considerable reabsorption from that area

Knisely That could easily be investigated

We have given frogs sodium sulphindigotate and this collects in the bile canaliculi and you can see the spaces in between granules of the secreted dye In all the portal spaces the internal contents of the bile tubules are stained a beautiful blue green or dark blue color You can see the granules in the canaliculi during life and you can see whether they are regularly spaced or coming closer together

Macgraith We thought of using tagged material and watching it come out in the blood

Figure 4 is just the ordinary India ink injection which I think very nicely shows this sinusoidal connection along the portal tract You will see it much better in some of the other specimens I have brought with me

So much now for the picture of the circulation in the portal tract We think that it is important to realize that there is this close



FIGURE 4 India ink injection of a rat's liver A branch from a moderate sized portal vein is supplying the continuous network of sinusoids lying at the periphery of the portal tract Reprinted by permission from *Ann Trop Med* 43 230 *(Plate V)* *(1949)*

connection between the arterial and venous side at any rate It is not too much to suppose that there must also be a certain amount of anastomosis there There is a considerable mixed arterial and venous plexus running in the loose tissue of the portal tract

Now getting away from the anatomy of the vascular arrangements in the liver we have been working as some of you may know for years on the possibility that the circulation through the liver can be controlled to some extent by active — reflex possibly — constriction of the hepatic venous tree There is for instance the sphincter mechanism of Bauer and Dale which occurs in dogs at the point where the hepatic veins drain into the vena cava We believe however that active constriction can occur at many points

in the venous hepatic tree beyond the central vein not only in the dog where there is known to be lots of smooth in the hepatic venous vessels but also in other animals in which Bauer Dale and their co workers(6) failed to find any sphincters e g the rabbit the rat and the guinea pig We have been able to show I think that constriction of even the smallest radicals of the hepatic veins can occur in these animals This constriction can result in a swelling and engorgement of the liver which is very similar in principle to the picture that you get in the whole liver of the dog in anaphylactic shock

In order to demonstrate this satisfactorily to ourselves we had to repeat the perfusion experiments of Dale and others and we rapidly realized that all of these workers had some time or other stopped the circulation through the liver while they got their perfusion going and that as a result of this because this con strictor mechanism is so very sensitive when they got their circula tion through the liver going again they were no longer putting their perfusion through a normal organ They were we believe putting it through an organ in which some of these veins had already constricted

And so my colleague Dr Andrews set out to develop a method of perfusion in which the liver (which was not removed from the animal) would at no time be short of oxygenated blood And by making sure that oxygenated blood was going through the portal vein when we were tying off the artery and vice versa we were able to produce a liver which was quite different from that described by Dale and other workers We have called this the red liver It looked exactly like a normal liver It was a nice liver reddish in color It was soft It lay flat back in the belly cavity and it had reactions to certain drugs which were different from those described by other authors working on the perfused liver

We found that when we stopped the circulation of the liver for 10 minutes or so the organ became darker in color with firm rounded edges It was in effect quite different in appearance from the normal organ In order to avoid the word "anoxia" we had described the first liver as red and the second one as blue and I thought you would be interested to see the very obvious differences between the effects of drugs on the red liver and on the blue

In the blue liver small doses of adrenalin produce an increased hepatic outflow and a diminished arterial and venous inflow and

a general reduction of liver volume In our red preparation adrenalin produces a different effect There is a considerable *reduction* in the hepatic outflow accompanied by a reduction in arterial and venous inflow and in the liver volume

Knisely What species is this one, please, sir?

Maegraith These are dogs

Knisely Anesthetic? Which one?

Maegraith The difficulty about anesthetics is almost insuperable We bleed the dog under as low a dose of nembutal and ether as we can and then leave him for some time with the lungs being aerated These preparations of ours will go on for hours without any trouble at all We found that if we used them very soon after nembutal, we got very bad effects as long as the aeration was carried out by the dog's lungs, but if you use an aeration machine you can overcome that difficulty

Knisely Did you say you bled the dog?

Maegraith Yes, this is perfusing the dog's own blood back to him

Sherlock At a constant rate?

Maegraith Well, yes You could vary the rate if you wished

Best With an anticoagulant?

Maegraith We used heparinized or defibrinated blood

Sherlock If the perfusion pressure was unaltered and the portal venous inflow was impeded, then the perfusion rate must have changed

Maegraith Oh, yes, that was varied from time to time

The first record, Figure 5, shows the Dale effect in blue liver, the adrenalin after injection into either the hepatic artery or the portal vein produces an increased hepatic output The second record, Figure 6, shows the diminution of output produced with a similar dose of adrenalin in the red liver If we allow the red liver to become blue the same dose of adrenalin will give the Dale effect (The effect of acetylcholine injection has been referred to in our presentation Differences in action in the red and blue livers are very obvious)

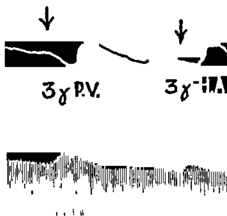
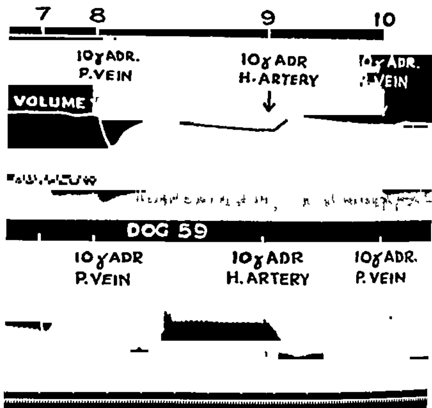


FIGURE
3γ of a
increase
portal v

Now, I think it is quite pointless to push this right on with all details, but may I just say this we regard this production of the red and blue liver and the differences in adrenalin effects as evidence that there is something on the far side of the liver which can interfere with the blood flow through it and which can change the immediate reactions of the organ to physiologically active compounds such as adrenalin and acetylcholine Unless you make quite sure your liver is in the red state you will probably not

than the blue one



Shorr Have you repeated perfusion experiments in the rat in which there is no well defined sphincter? I wondered how much influence you attributed to the hepatic sphincter?

Maegraith No, we have not done this in the rat but we repeated Weil's experiments(2) in rats and some other animals, which showed a similar effect You know, Weil sensitized dogs to serum and then, instead of injecting the trigger dose intravenously and getting the well known rapid swelling of the liver, he injected a tiny dose of antigen straight into the liver substance, and in the area where he put it he got tremendous congestion and the picture

that he would have got in the whole liver had he put in the trigger dose

Best Is this in the rat?

Maegraith No this is in the dog

We have obtained similar results in the rat the guinea pig and the rabbit

Best You dont get any massive swelling in other species than the dog do you?

Maegraith You do get noticeable swelling in some of them You dont get anything like the swelling that you get in the dog but you get a very definite congestion with the same sort of histological picture that you saw in that pig

Hill Is it blood congestion? Any edema or exudate?

Maegraith No it is nearly all blood It comes up in the course of a half minute

Watson Have you studied the histamine effect?

Maegraith We have only just got on to histamine (Large doses appear to have much the same effect as acetylcholine)

Popper Do you assume that the necrotic lesions seen in your pictures of blackwater fever and malaria are produced by a constriction of the central and hepatic veins?

Maegraith Probably not the central vein I think there is probably a constriction somewhere in the hepatic venous tree beyond the central vein I think that interference of this part with the circulation of the liver has something definitely to do with the production of the lesion but I should not like to say exactly what the mechanism is

Popper I believe one sees a similar picture in toxic hepatitis due to other etiologies also This hemorrhagic necrosis is rather characteristic It occurs in the center of the lobules just outside the central vein or the branch of the hepatic vein both of which appear very narrow This is in contrast to the typical picture of passive congestion in which the central necrosis is associated with marked widening of the central vein This may answer the objection which Dr Knisely has raised because in both instances the toxic hepatitis and the congested liver the examination has been done on fixed post mortem specimens

Knisely We can learn valuable things from that I did not mean to damn the sections – of course we can learn valuable things from them

Popper But you are questioning the significance

Knisely How to interpret it

Popper We teach that the width of the hepatic veins is a characteristic difference between toxic hepatitis and passive congestion Wallach and I(7) considered especially important the observation that narrow venules piercing the wall of the branches of the hepatic vein – the outlet veins as Dr Knisely would call them – as indication of toxic hepatitis whereas in passive congestion these venules are not piercing but represent wide sinuses If you found the former in your specimen it would very much support the findings just presented

Turner Is this blueness irreversible?

Maegraith It is sometimes reversible This is very crude but if you get hold of the blue liver and knead it you can push out a large quantity of blood Some of the color comes back and it takes quite a long time before it swells up which suggests that there is an obstruction to the outflow

Shorr How long a period of anoxia is required to get a blue liver?

Maegraith Well I am not sure sir whether anoxia" is the right word because we have measured the oxygen content of the blood going in and there does not seem to be any very close relationship between the fall off in the oxygen content and the production of the blue liver except at very low oxygen tensions That I think fits in rather with the ordinary takeup of oxygen by the tissue cells You have got to have very low tension before you get any drop in your curve

Shorr How do you produce the blue liver then?

Maegraith The best way to produce the blue liver is to make a bad preparation do something wrong at the beginning and get your circulation mixed up Then the thing goes blue for some reason or other and stays there

Shorr Over a period of how long?

Maegraith If you have a red preparation and you want it to go blue you don t need more than a quarter of an hour Either stop the circulation or circulate anoxic blood

Fremont Smith It is true though you hesitate to use the word "anoxia" that when you produce acute anoxia you get a blue liver

Maegraith Well yes but that is different from anoxia

Fremont Smith Do you think that a reduction of blood flow through or into the liver is an essential aspect of producing the blue liver?

Maegraith Yes I do

Knisely Gentlemen it cannot be ischemia if it is blue Livers without blood are relatively transparent or yellow It is much more nearly anoxia than ischemia

Fremont Smith It is rate of delivery to the liver

Maegraith It is the slowing down of the rate of circulation into the site

Sherlock Am I right in believing that in the case of the red liver the giving of adrenalin results in an increase in capacity of the splanchnic area? If there is a diminution of output there must be an increase in the capacity of the splanchnic area If the per fusion rate is constant the thing must swell if the hepatic vein constricts

Maegraith It does not go quite like that In the red liver you get a reduction in inflow a reduction in the total volume and a reduction in the output — a reduction in all three of them

Sherlock So the flow must go down

Maegraith Well it depends upon which goes down most I suppose

Sherlock If you have constriction through the entire splanchnic area you cannot help but have diminished blood flow If adrenalin in the normal red liver produces a vasoconstriction throughout you would expect flow to diminish and we have shown in man who we suppose has a red liver that adrenalin in physiological doses increases blood flow through the splanchnic area(8)

Fremont Smith Including the liver?

Sherlock Oh yes the entire area

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O Leary(14) One would not, of course, from present knowledge and attitudes, expect the pancreas to be much of a blood reservoir But the precise details of when and how much blood is permitted to pass through the Islets of Langerhans may be rather complicated and certainly most important The insulin produced by the Islets can, as far as we know, get into the general blood stream only by being carried into blood flowing through the special capillary nets of the Islets of Langerhans The precise control of the rates of flow through each of these networks may be rather complex

To return to the control of the flow of blood through the intestine, the small veins of the intestine can change capacity And what may be surprising, there is an arterio venous anastamosis across the root of almost every intestinal villus This was studied by Spanner(15) Jarisch and Ludwig(16) studied the portal vein bed as a blood reservoir, finding that it had a very large capacity But as far as I know, no one has yet considered what kind of changes might be caused in circulatory dynamics by the opening of large numbers of the arterio venous-anastomoses which Spanner found as shunts across the roots of intestinal villi One would guess that such shunts would if open, permit arterial blood to be jetted into the intestinal roots of the portal vein but this is only guessing What we need now is more precise experimentally derived information concerning the neuromotor hormonal and possible pharmacological control of these shunts This information should be obtained from experimentation upon several species of animals Additional information concerning the anatomy or architecture of such vessels can almost certainly be obtained from human biopsy material or from the intestines of persons who had died a sudden death

The spleen can certainly store and release concentrated ' cells in many species of animals including human beings (references quoted on page 51 of Knisely Bloch and W these references begin with the original papers by B ie year 1921) There is very precise control of each ood vessel within the spleen(18 19a,b)

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Maegraith Of course, it is difficult in a perfusion to argue about the splanchnic area, because you have got most of it cut off We are dealing with an extirpated liver I think it might be different if you had the splanchnic area tied up with it *

Smetana What is the histologic difference between the red and the blue liver? Is there any necrosis in the blue liver?

Maegraith Yes, you get necrosis Perhaps you read the recent article in the *Lancet* by Delorme(9) on the production of necrosis *in the liver by perfusion You can get necrosis in five hours by* perfusing the organ with the femoral venous blood We have found a certain amount of necrosis in the blue liver, too

Knisely Dr Link said something here a while ago that I want to refer to because of its profound wisdom He said the question is *not who is right or who found it first, the question is to try to find* out how much of this we can understand as we come to it

The language used here today is largely the language of gross physiology People are thinking in terms of the whole gross splanchnic area However the whole portal reservoir system consists of at least three main separable parts which work in cooperation with each other like members of a team

There is one part made up of all the small veins which drain the intestine these all working together have a finite total capacity *And there is the spleen which has a total capacity and which has* its own vasomotor control mechanisms And then there is the liver itself each lobule of which has a whole series of special contractile *structures*

Also the pancreas has its own internal circulation which has scarcely been studied yet and certainly has not been studied to see if it can change capacity For references on pancreas circulation see Wharton(10) Berg(11), Beck and Berg(12), Covell(13) and

* ... ments on m n The dose

... reactions are not quantitatively the same as ... e asked Dr Bradley

Maegraith

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portal anastomoses, the terminal branches of the intrahepatic inter lobular portal veins, the inlet sphincter located at the point where the afferent end of a sinusoid joins a portal vein Also the entire length of the sinusoid linings also the outlet sphincter, and the central vein of the lobule, and probably the sublobular veins and other large hepatic veins in most species

Dr E H Bloch found out a while ago that when adrenalin in a dose of say 1 10,000 000 was put on the outside of frog liver the small hepatic outlet sphincters begin to shut off And I think that a dose of approximately 1 100,000,000 similarly placed was followed by the opening of hepatic sinusoid outlet sphincters The point for the moment is that differences in concentration of stimulating substances which strike the specific contractile structures may make great differences in the responses which follow This is something to worry about

Thomas and Essex(20) show that the veins of dogs can contract powerfully in response to special stimulation, they present an excellent review of most of the older literature

Present day investigators are apt to forget that the late Professor August Krogh demonstrated that the livers of human beings can and do act to store and release considerable volumes of blood (21a,b,c) The experiments were performed upon the unanesthetized investigators themselves and clearly showed that the livers of human beings could store and release blood

There are portions of the older literature describing experiments in which men failed to find that the livers of various species of animals could store and release blood Some of the literature says that outlet valve mechanisms or Sperre" mechanisms do not exist in certain *species* In general I now believe that these ideas were based upon a failure of the experimenters to realize the meaning of negative evidence

Professor Robert R Bensley taught the meaning of "negative *evidence*" as follows He said, "If you have a frog and you poke him with a needle and the frog hops you then can conclude 'a frog can hop', but if you poke the frog and he does not hop that *is negative evidence* You do not know whether you found that a frog cannot hop or failed to find that a frog can hop"

Now, experimenters applied stimuli to dogs or isolated dog livers and got the outlet valve mechanisms to contract And that was

positive evidence Then when experimenters took similar stimuli and applied them under what seemed like similar conditions to other species of animal they sometimes or often failed to get responses from the livers

One reason is perhaps that in a large animal such as a dog or goat quite a bit of blood can be lost at surgery and quite a bit of blood still remains which can be stored in the liver when the stimulus is applied

However when surgical operations are done on small animals such as guinea pigs or cats the loss of blood which may appear to be rather small in total amount may actually percentage wise be high enough so that the animal contains little blood which can be stored in the liver even if the outlet mechanism does contract If one determines the presence of the outlet sphincter mechanism by seeing the liver fill then failure of sufficient blood to fill it will give failure of the experiment This I believe to be a possible large source of error in the failure to find hepatic outlet valve mechanisms in the livers of small animals

There is another factor Histamine causes the outlet valve mechanisms to close in the dog(6) That must not be taken to mean that the same chemical stimulus must necessarily cause these outlet valve mechanisms to close in every other species There is the problem of comparative pharmacology The histamine which one buys in a bottle is apt to be but one type of histamine and according to Professor Koch who taught me biochemistry there may well be many "histamines" Further histamine may not be an adequate stimulus that is adequate for initiating the closure of the hepatic outlet valves in species other than dogs

In summation the great portal reservoir consists of at least three parts the veins of the intestine and the larger branches of the portal vein the spleen perhaps the pancreas and certainly the liver itself Hepatic outlet valve mechanisms capable of causing the liver to store and release blood have been found in a number of species including human beings Experiments which have failed to find such mechanisms in some species have I believe been based upon failure to recognize the nature of negative evidence It will be necessary to study the anatomy physiology and pharmacology of all possible contractile structures between the hepatic sinusoids and the openings of the large hepatic veins into the vena cava in each of a series of species before our general knowledge of this set of structures is complete

I find it much easier to believe and this is certainly wishful thinking that all vertebrate species have mechanisms which control the outflow of blood from the liver than to believe that some species have them and that some do not have them

Best I should think that Dr Maegraith's preparation might be a very good one in which to demonstrate an anaphylactic reaction in an isolated perfused liver I was working in London in Dale's laboratory when Walter Bauer and Dickinson Richards were working with Dale on this problem and they were not able to demonstrate an anaphylactic reaction in the liver from a sensitized dog when they added the antigen Later Jaques(22) and Rocha e Silva(23) working in my laboratory were at least partially successful and got the liberation of huge amounts of both histamine and heparin And I think it was largely the better circulation of the liver For a while they thought it was the presence of the red blood cells and absence of anticoagulant and so on but it turns out that it is probably just an improved circulation better oxygenation of the blood for a physiologic condition of the liver

I thought it would be interesting to refer just a moment — and I should restrain myself because I want to restrain all of us now — Rocha e Silva has repeated an old observation of Bordet on anaphylatoxin He makes a fraction by Bordet's procedure from normal blood and adds this to the perfused guinea pig's lung and gets an immediate outrush of histamine He thinks Bordet's anaphylatoxin which everybody has forgotten is a true bill and he can make it from normal blood

Campbell In the hemorrhagic necrosis of blackwater fever your illustrations have been of centrilobular necrosis which obviously would fit quite nicely with such an explanation as you suggest though one could I suppose fit it in with an explanation based on impairment of the incoming blood flow in the periphery of the lobule either vasopastic or associated with general hypotension But have you noticed other types of focal necrosis paracentral or haphazard or eccentrically placed in the lobule?

I have been very interested recently in cases of transfusion reactions with distal nephrosis and in these as everybody knows focal necrosis in the liver is not uncommon In my experience it has usually been of the type you illustrate in blackwater fever but from time to time one sees what one might call paracentral or eccentric necrosis which seems to be not explained by central

hepatic venous constriction It might I suppose be explained by constriction of the central ends of *some* of the sinusoids It would seem to me perhaps more easily explicable by a disturbance of the incoming blood in the periphery of the lobule I put it down in my own mind to arteriolar vasospasm which would explain the eccentric location I have seen it also in a case of cortical necrosis of the kidney which I think many of us nowdays would be willing to call a renal arteriolar vasospastic lesion thereby supporting the idea that the liver lesion is an arteriolar vasospastic type of necrosis

In view of your pictures and what Dr Knisely has told us it might also of course be a spastic affair in some of the small branches of the portal vein coming in at the periphery of the lobule But however it is caused it seems to me that it does occur and that it is a type of necrosis which has a different pathogenetic mechanism from the usual centri lobular necrosis

I would be very interested to know if you have seen that type of eccentric or paracentral necrosis in blackwater fever

Maegraith No I have not There is sometimes some overlapping of the lesion from one lobule to another in malaria

I realize that the value of the work we have done if anything at all lies not so much at the moment in our interpretation as in the fact that it directs our interest back again to the vascular mechanisms of the organ and emphasizes what I said earlier that the vascular mechanism of an organ is as much a specific part of that organ as any other part Whether you think we have made a good case for our interpretation or not does not really bother me very much It has taken us the best part of three years to get this perfusion to work and it has only been in the last six months that we have really been able to say on Thursday 'We will have a red liver today and sometimes get one

Fremont Smith I should like to say one word if I may because it seems to me the discussion brings out several points

I was thrilled by what you brought us and I think it is wonderful that one can have a red liver on Thursday I also liked so much what you said in your opening remarks that it is extremely difficult to repeat a biological experiment I think that is one of the funda mental truths that we keep forgetting and I should like to touch just a bit on the relativity of facts

The fact is that we are still bound to the concept of unicausality We still look for the response to adrenalin as if there was a

response to adrenalin We are still caught in the old belief that you get a response as a result of a stimulus, and that the response is characteristic of a stimulus We really know that there is no response which is characteristic of a stimulus It is only characteristic of the stimulus under the given circumstances, the total environment *Gestalt* in which the organism exists, and that you can get and normally and characteristically do get diametrically opposite responses to the same stimulus in the same organism if the conditions are set rightly We know that for adrenalin, we know it for pitressin, which is either a diuretic or an antidiuretic at will, depending upon the state of the organism Surely, it was always thought of as a diuretic, because it was always given to anesthetized animals, and then it became recognized as an anti duretic when it was given to unanesthetized animals in water diuresis And then Walker(24) found that you could come back again and call it respectively a diuretic in unanesthetized animals provided you dehydrated them Well, that is one illustration and one can build them

I find it necessary to remind people — remind ourselves — of that, because we are unconsciously looking for the characteristic response to a characteristic stimulus It seems to me that this discussion brings out a little anxiety that we have if we cannot find the thing always clicking What we really need to do is with great care, specify the conditions under which we are operating for any given stimulus and response, with the expectation that that response will only be characteristic of that stimulus under those circumstances, and that quite different quantitative or qualitative response can be expected from the same stimulus in the same organism under different nutritional, psychological and anesthesiological circum stances

For instance, possibly the humans that you were studying were not anesthetized

Sherlock They were unanesthatized

Fremont Smith Whereas all the animals had anesthesia It is possible that that might be one of the discrepancies

And the discrepancies really are the clues to the next bit of understanding that you were speaking about, Dr Kinsely All advances are made by attention to discrepancies

Best We must go on at this point I shall ask Dr Sherlock to speak on 'The Effect of Insulin on the Liver in Normal and Diabetic Man'

THE EFFECT OF INSULIN ON THE LIVER IN NORMAL AND DIABETIC MAN

SHEILA SHERLOCK, A G BEARN
AND B BILLING
Postgraduate Medical School
University of London

WE HAVE USED the technique of hepatic vein catheterization The catheter is introduced into an antecubital vein and passed via the superior vena cava and right auricle into a branch of the right hepatic vein We are now in a position to sample blood from the liver The bromsulphalein extraction technique is used to estimate the total hepatic blood flow

I must remind you of what Dr Knisely has already remarked this morning With this technique we can only measure the metabolic changes occurring in the entire splanchnic area The difference in the concentration of a metabolite such as glucose between arterial and hepatic venous blood will be due not only to hepatic activity but also to the spleen, intestines and other constituents of the so called splanchnic area In the fasting state one can assume that these latter organs play little part in the production of the metabolic changes I am going to describe

If we take the difference in glucose concentration in hepatic venous and arterial (or capillary) blood and multiply it by the hepatic blood flow obtained by the bromsulphalein technique then the absolute amount of glucose leaving the liver per minute is obtained(25)

Hepatic glucose output mg/minute = (Hepatic venous glucose conc mg/100 ml − capillary glucose conc mg/100 ml) × hepatic blood flow ml/minute

We can use this technique simply to decide whether the primary defect in carbohydrate metabolism in diabetes is mainly a hepatic one or mainly in the peripheral utilization of carbohydrates Here are the two current views (Figure 7) One view is that the blood glucose rises in diabetes because the peripheral utilization of glucose is impaired but liver glucose production is normal The other one, quite popular in the Middle West, particularly elabo-

So that by and large there is no evidence of overproduction of glucose by the liver in diabetes I am going to modify that state ment a little later

Best Are these diabetics that have had no insulin for some time?

Sherlock No sir Of these diabetics 19 had never had insulin and the remainder had been off it for 48 hours They had all previously been on a high carbohydrate diet

Best So 19 of them were mild diabetics

Sherlock Well I think on a high carbohydrate diet diabetics will get by for a few days without insulin The diagnosis of diabetes had just freshly been made in these 19 subjects and this was their first admission to hospital for stabilization

Sborov What state of fasting?

Sherlock Twelve hours fasting

The results of the effect of insulin on hepatic glucose output in 15 normal subjects have been measured (Figure 8) In the fasting state the hepatic venous glucose concentration exceeds the capil lary (or arterial) glucose concentration by about 14 mg/100 ml When the difference is multiplied by the hepatic blood flow the hepatic glucose output is obtained and this as we have seen is of the order of 100 mg/minute quantities After a control period of 40 minutes soluble insulin is given in a small dose 0 1 unit per kg intravenously The effect we notice first is that the hepatic venous glucose concentration drops The capillary glucose concentration drops too but not so much So that the hepatic venous – capillary glucose difference diminishes as a result of the insulin In fact in 9 of these 15 observations the capillary glucose level actually exceeded the hepatic venous one indicating that as a response to

apparent but in 9 of the subjects the actual amount of glucose entering the liver was greater than the amount leaving In other words insulin administration resulted in an actual uptake of glucose from the circulation

This drop in hepatic glucose output occurs within 2 minutes and it seems that insulin apart from other action does have a very prompt effect on hepatic glucose output

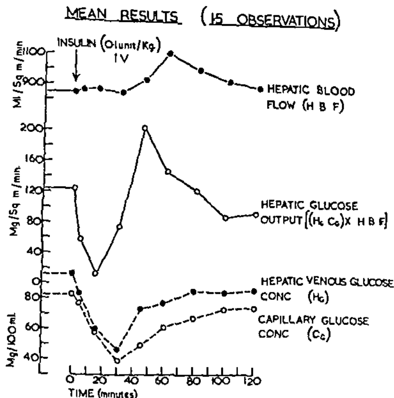


FIGURE 8 The effect of insulin on hepatic blood flow and hepatic glucose output in normal subjects

The hepatic glucose output drops and the capillary glucose level after about 30 minutes has reached values of about 40 mg/100 ml, and the subject experiences hypoglycemic symptoms There is restlessness, sweating, tachycardia, rise in arterial pulse pressure, and so on

I should like to draw your attention now to the mechanism of the recovery of the capillary glucose concentration The obvious explanation is that adrenalin is released, and I think there is no doubt that epinephrine or adrenalin is released as a result of hypoglycemia There is plenty of supporting evidence in the literature to support that statement The increase in the hepatic *blood flow* that occurs 45-60 minutes after the insulin was given is almost certainly an adrenalin response We have shown that adrenalin does increase hepatic blood flow (8) At the time of increased hepatic blood flow after insulin there was an increase in blood

lactic acid and pyruvate concentrations other well known adrenalin effects

So that part of the mechanism by which hepatic glucose output restores the blood sugar to normal is due to adrenalin but it is rather interesting that the recovery of the hepatic glucose output preceded the hepatic blood flow increase The rise in hepatic glucose output precedes the hepatic flow increase by about 15 minutes There may be some other mechanism at work here, so that the recovery of blood glucose after insulin shock is not only due to the release of epinephrine but it could also be due to some other preceding factor Well your guess is as good as mine as to the nature of this factor One might invoke the alpha cell hormone from the pancreatic islets which is known to be directly glycogenolytic on the liver but I have no direct evidence on that point I know Soskin(29) believes the liver has an inherent homeostatic property which maintains blood sugar He may be right that the liver has some power some property inherent in the liver cells themselves by which the arterial glucose is maintained at a normal level apart from any accessory hormonal influences

Best Why couldn t it be just using up the small dose of insulin?

Sherlock The results in diabetes are going to be quite different and the action of insulin much more prolonged even though presumably the rate of destruction of insulin is much the same in normal subjects as it is in diabetics

Best I see

Sherlock And it is only 20 minutes before hepatic glucose output starts to recover

Best You gave a very small dose

Sherlock About 7 units intravenously

Shorr Would it be possible to approach this by using a sympatholytic drug such as dibenamine?

Sherlock Yes, I thought of that and I think the answer is shown in the next observation because the diabetics don't become hypoglycemic and hence do not release adrenalin yet they also recover their hepatic glucose outputs to preinsulin values This suggests that the liver has an inherent mechanism for maintaining the blood glucose level

Fremont Smith What about a continuous intravenous smaller dose of insulin so that you would know you had a continuous level?

Sherlock That I think we ought to do

Stetten This is English insulin you are using? Was it Boot's or Lilly's

Sherlock Yes, we are using an English insulin, made by Boots

Stetten There is a difference in the glycogenolytic factor in them Was there any evidence of that?

Sherlock We got some of the Novo Insuline from Denmark, known to be almost free of the glycogenolytic factor and it gave exactly the same results as I have shown The British insulin, apparently, does not contain this factor Anyway, this very immediate drop in hepatic glucose output would show, I think, that it probably did not

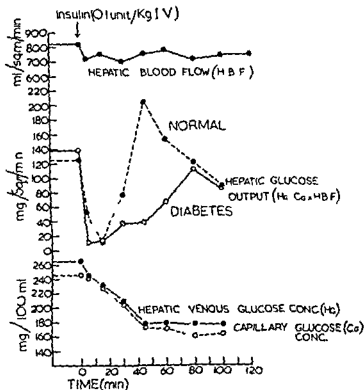
Neefe These are normal subjects?

Sherlock Yes The results of diabetics are shown in Figure 9 I emphasize that these are a mixed bag of diabetics (I am going to break them down in a minute into various clinical types)

The hepatic venous capillary glucose difference in the fasting state *is not significantly different from that found in the normal* subjects The hepatic blood flow does not differ either, so that by and large these diabetics have a normal fasting hepatic glucose output Intravenous insulin results in an immediate drop of the hepatic venous glucose concentration and a smaller drop in the capillary glucose concentration, hepatic blood flow is unchanged so the insulin has, as in the normals, caused a diminution in hepatic glucose output The extent of the diminution is much the same as in normals There are two differences from the normal subjects The depression of hepatic glucose output after insulin in diabetics *is much more prolonged and the hepatic blood flow is virtually* unchanged over the two hours of the observation

The lowest blood glucose reached is about 160 mg/100 ml These diabetic subjects never become hypoglycemic They never show epinephrine release and, naturally, hepatic blood flow does not rise

Shorr Should one other possibility be considered, namely, the extent of storage of glycogen in the liver?



DIABETES mean results (33 observations)

FIGURE 9 The effect of insulin on hepatic blood flow and hepatic glucose output in diabetes mellitus Reprinted by permission from Sherlock S Hepatic vein catheterization in clinical research *Proc Inst Med (Chicago)* 18 335 (1951).

Sherlock I do not know whether the diminution in output of glucose from the liver due to insulin represents a storage of glycogen There is a great difference in the extent of this diminution of hepatic glucose output in the various clinical types of diabetes I am coming to that directly The findings shown in Figure 9 are derived from the average results for 33 diabetics This clearly does not show the scatter of results

How can we compare the "hepatic insulin sensitivity" in the individual cases? We have done this by measuring the effect of insulin on the output of glucose from the liver at the end of 30 minutes If the area between the curve of diminished hepatic glucose output and the fasting glucose output is measured with

a planimeter the actual diminution in hepatic glucose output can be calculated This is an absolute quantity measured in grams (Figure 10) This amount can be used to compare the results in diabetics and in normal subjects I have termed it 'hepatic uptake" I must explain this, it is not an actual uptake but rather a diminution in hepatic glucose output It is the amount by which the liver has diminished its glucose output as a response to insulin and is not necessarily the same as the amount of glucose taken up from the blood stream

The hepatic glucose uptake in normal subjects is remarkably constant (Figure 11), 90 per cent of 12 subjects have an uptake between 2 and 6 grams The values for the diabetics are much more variable 30 per cent of the 33 subjects diminished their hepatic glucose output by less than 2 grams and 37 per cent have values greater than 6 grams (Figure 11)

Best But your concentration of insulin in the two series would not be the same, would it?

Sherlock We compared them at 30 minutes so that the effects of hypoglycemia which I have already described in the normal subjects would not need to be considered

Best Yes I know but if we knew the concentration of insulin in the nondiabetic liver, and you have added a certain dose to that — then in the diabetics, who have presumably less, the con-

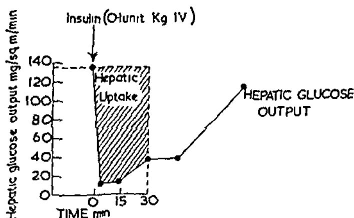


Figure 10 Diabetes shaded area represents the amount in grammes by which the liver diminishes its glucose output for 30 minutes after intravenous insulin and has been termed the "hepatic uptake"

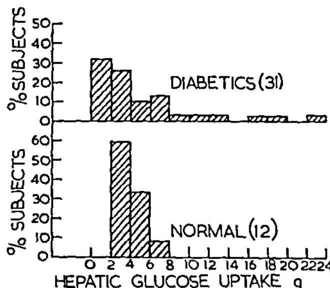


FIGURE 11 The hepatic uptake of glucose for thirty minutes after insulin in normal subjects and in diabetes Reprinted by permission from Sherlock S Hepatic vein catheterization in clinical research *Proc Inst Med (Chicago)* 18 335 (1951)

centration of insulin in the liver is less presumably than in the normal

Fremont Smith You mean even after the insulin is added?

Best Yes

Sherlock You mean compared with normal subjects diabetics have different insulin contents in the liver?

Best I would suspect that they do but I dont know of course

Sherlock Anyway there are clinical differences between the diabetics with large hepatic glucose uptakes after insulin and those with low ones The high ones are young and they do not have cardiovascular complications and they are unstable in terms of readily going into hypoglycemia or ketosis whereas the group with low uptakes are usually old obese often have cardiovascular complications or gangrene and rarely manifest either hypoglycemia or ketosis I can demonstrate that by using examples from the two groups

I will start with a subject with a high hepatic uptake (Figure 12) The diabetes had just been discovered and the patient had

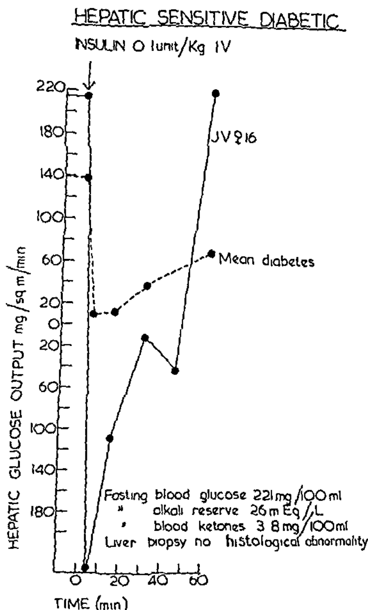


FIGURE 12 The effect of insulin on hepatic glucose output in an hepatic sensitive diabetic The dotted line represents the mean of results obtained from observations on 33 patients

never had insulin This girl is an hepatic insulin sensitive diabetic She diminishes her hepatic glucose output as a response to insulin considerably more than the mean for the whole 33 observations on

diabetics or normal subjects. Her actual diminution in output is about 12 grams for the 30 minutes after the insulin was given. She was mildly ketotic—blood ketones 3.8 mg/100 ml. (normal up to 2 mg. per 100 ml.) Liver biopsy showed a normal complement of glycogen and no cirrhosis or fatty change. Histological abnormalities in the liver are never detected in this group. The more acutely diabetic like this patient do perhaps have a rather high fasting output of glucose from the liver. I shall discuss that later.

The next example is of a different type of diabetes. The hepatic insulin insensitive one (Figure 13). This patient diminishes her hepatic glucose output after insulin considerably less than either the mean decrease for diabetics or for normal subjects. Moreover the diminution is not well maintained. Her actual diminution is about 1.2 grams for the 30 minutes after insulin was given. She is grossly over weight, not in ketosis and it would be difficult to make her acidotic.

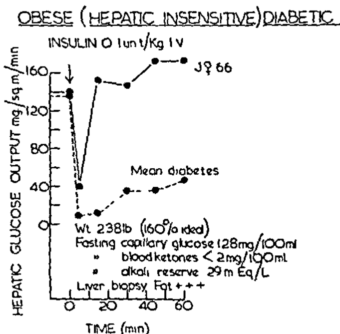


FIGURE 13. The effect of insulin on hepatic glucose output in an hepatic insensitive diabetic.

Best If I may interrupt — I think this is fascinating — Dr Wren shall in my department has analyzed the pancreas from these two types of cases He has done about 100 now He would find 50 per cent of the normal insulin content in the pancreas in this case, one could predict, and zero in the other case you showed And the blood levels if we can take Lawrence and Bernheim s figures would be appreciable in this case and zero in the case you showed previously

Sherlock Liver biopsy sections in this case show that there is, histologically, 3 plus liver fat increase over normal (Figure 14) We found a correlation between the amount the liver diminishes its hepatic glucose output after insulin and the amount of fat in it

Hill What is the cellular infiltration in that photomicrograph?

Sherlock I think that is within normal limits It is not very marked

Hill What is the age of this patient?

Sherlock Sixty-six

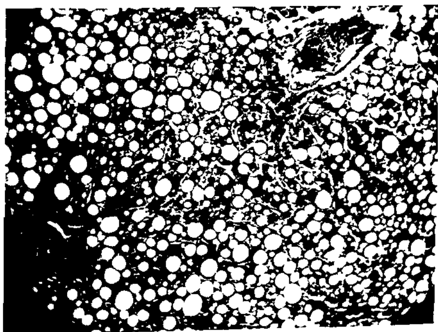


FIGURE 14 Hepatic insensitive diabetic Same patient as in Figure 7 Liver biopsy sections show 3 + fat Stained H E x 145

Sborov. Was there any other evidence of liver disease besides the biopsy?

Sherlock. No

Gyorgy. Liver function tests?

Sherlock. Bromsulphalein quite normal. This is the point I want to make. If, in diabetics, the histological assessment of liver fat is compared with the hepatic glucose uptake after insulin then a positive correlation is seen (Figure 15). The point is that the more fat there is in the liver, the less will the liver diminish its glucose output as a response to a standard dose of insulin. I think that is very significant.

There are many reasons why this should be so. I do not think the explanation is mechanical. I cannot really believe that the liver is so full of fat that it cannot take up glucose. One association may be with body weight, for many of these diabetics are obese.

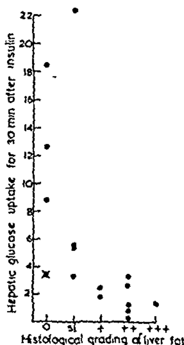


FIGURE 15. The relation between liver fat and diminution in hepatic glucose output after insulin. X indicates results for a subject in severe ketosis.

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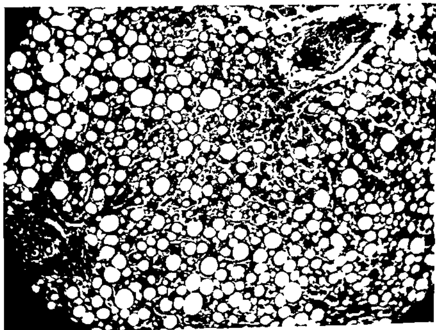


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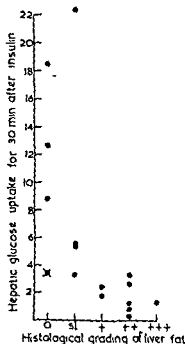


FIGURE 15 The relation between liver fat and diminution in hepatic glucose output after insulin X indicates results for a subject in severe ketosis

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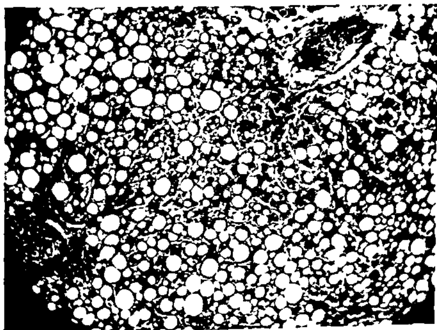


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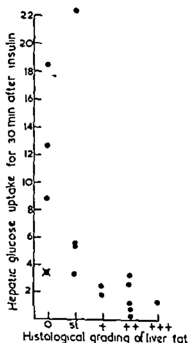


FIGURE 15 The relation between liver fat and diminution in hepatic glucose output after insulin X indicates results for a subject in severe ketosis

Comparison of the body weight in control subjects having normal glucose tolerance tests with the amount of histological fat found by liver biopsy shows that there is a positive correlation i e the fatter the patient the more fat there is in the liver (Figure 16) An example of such a fat woman is shown in Figure 17

Gyorgy Thats right Redundant in every respect

Sherlock She makes pies Dr Gyorgy

Gyorgy She eats them too

Sherlock Liver biopsy sections showed 2 plus of fat (Figure 18)

Hepatic vein catheterisation studies show that the fasting hepatic glucose output is normal After the standard dose of insulin the diminution in hepatic glucose output is of the same degree and duration as for the mean for normal subjects (Figure 19) If a

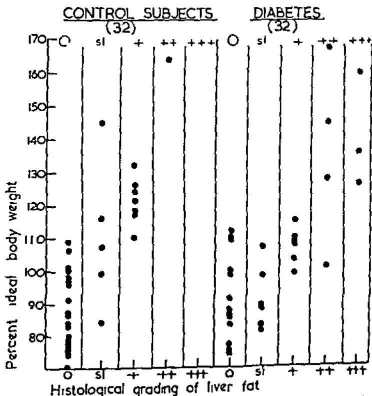
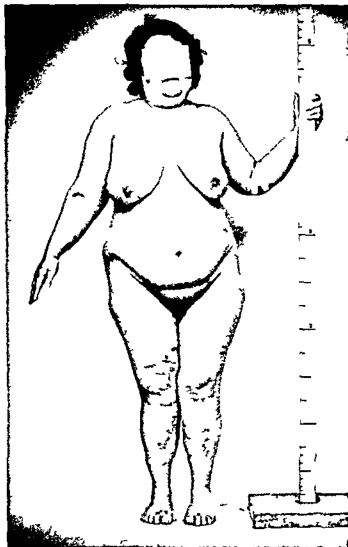


FIGURE 16 The relation between body weight and liver fat in normal and diabetic subjects



F GURE 17 Obe e subject F H

person is over about 110 per cent of their ideal bodyweight they get fat in their liver

Best That is irrespective of how much insulin you give them?

Sherlock These are norm il subjects

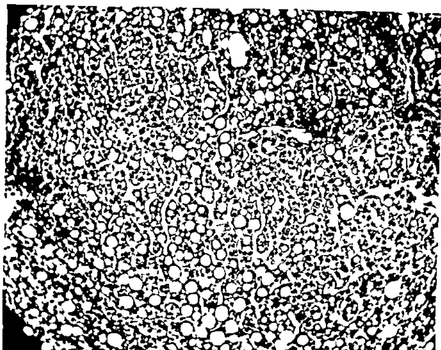


FIGURE 18 Same patient as in Figure 10 Liver biopsy sections show 2 + fat H E x 145

Sborov Were carbohydrate tolerance tests done?

Sherlock Yes

Sborov And they were normal?

Sherlock Yes *Oral glucose tolerance tests were normal* Liver function tests were normal also I have not done Himsworth areas on all, but such Himsworth areas as I have done are normal

Best What are Himsworth areas?

Sherlock The area between the curve of the glucose tolerance test and the glucose insulin test is a measure of insulin sensitivity

Best Oh, yes

Sherlock Now, the diabetics also show fat in the liver I don't know whether I can convince you of it, but they show relatively more fat than do the controls For instance although I was able to find several of my controls with 1 plus, I only managed to find one with 2 plus, and that was the patient (Figure 17) who is a

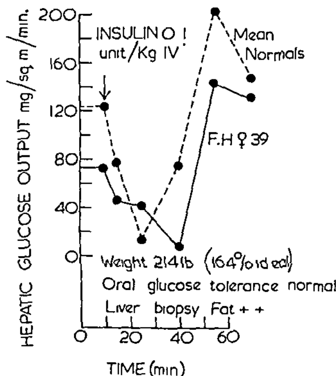


FIGURE 19 The effect of insulin on hepatic glucose output in an obese non diabetic subject (subject M H)

grossly overweight woman, whereas in the diabetics I had no difficulty in finding 1 pluses at relatively low body weights, and found many more 2 pluses and 3 pluses than in the normal subjects

Sborov Any cirrhosis in that group?

Sherlock No sir Liver biopsies did not show fibrosis

Hill Did you ever do a serial biopsy in any of these to see any changes – in these 2 pluses and 3 pluses – to see whether the liver fat was dynamic and moving? Were these all single biopsies?

Sherlock Are you talking about the sampling error in the aspiration liver biopsy specimens?

Hill Yes

Sherlock Oh, yes, I am sure there is an error That is why you have to do a large series of 32 cases

Hill Then the next thing on each case you did just one biopsy?

Sherlock Yes

Hill You did not repeat it again It may be that the fat content may change from day to day

Sherlock Yes that is true but unlikely

Hill I am trying to keep to the dynamic aspects

Sherlock I don t think the histological grading of fat could ever be dynamic Microscopic appearances are not very vital

Hill What you see with the microscope today is different from what you saw yesterday

Best You want a continual viewing apparatus

Neefe You did the biopsy after what period of fasting?

Sherlock Both groups were on the same sort of diet and had been fasted 12 hours

It seems then that part of the increase in liver fat in diabetes is just associated with obesity but I feel there is something else as well and I can only just speculate Speculating I feel that the pituitary and its fat mobilisation factor could be involved But increased fat in the liver does occur in diabetics

Another factor influencing the hepatic response to insulin is severe ketosis This is illustrated in Figure 20 The patient is severely ketotic and the diminution in hepatic glucose output after insulin is much less than the mean for all the diabetics These ketotic patients will revert from being hepatic insulin insensitive to being hepatic insulin supersensitive if the ketosis is controlled so that severe ketosis will diminish the response of the liver to insulin but in these subjects liver biopsy histology is – I should not perhaps say normal it is a bad term – I should say not remarkable contains no fat and the glycogen content is normal Earlier work has shown that the hepatic glycogen content is quantitatively normal(30) There is a positive correlation which is statistically significant between the amount of glucose put out initially from the liver and the amount by which this output is diminished upon being given insulin

This enables me to modify my original statement that there is no overproduction in diabetes Our feeling is that most diabetics

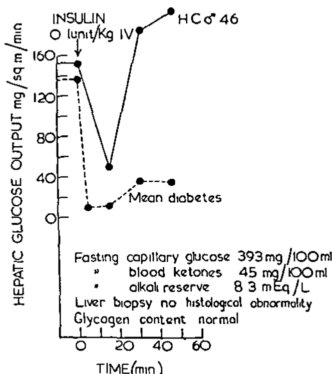


FIGURE 20 The effect of insulin on hepatic glucose output in a ketotic diabetic

do not overproduce glucose in the liver, but if they are becoming out of control and becoming ketotic, they do overproduce, and as a result of that they have a better response to insulin Then as they go into frank coma, it appears that the hepatic glucose output drops and the response to insulin drops, too But we have very few observations of people in coma – I think only three – and I should not like to be too dogmatic about it But our feeling is that the average diabetic without insulin does not overproduce but that the sensitive one does tend to produce too much glucos in an attempt to improve his hepatic sensitivity, and then if eventually goes into frank coma, this response is again diminis

In diabetes the ability of the tissues to use carbohydrate is impaired and the head of pressure of blood sugar is raised until utilization is adequate At one time the hepatic glucose output must have been increased but once the capillary glucose level has reached a sufficiently high value, equilibrium is maintained in which liver production is normal and peripheral utilization is normal too, provided the blood glucose level is high (Figure 21)

I agree with Dr H P Himsworth that "The rate of utilization of carbohydrate by the tissues is governed by the height of the

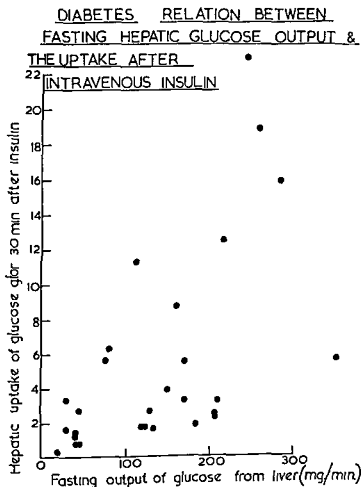


FIGURE 21 Diabetics and normal subjects relation between fasting hepatic glucose output and diminution in output after intravenous insulin for 30 minutes

blood-sugar and in any particular circumstances this is adjusted to the level which comes nearest to ensuring an adequate rate of utilization "(31)

In the uncontrolled diabetic — I put "controlled' in quotes, I don't really mean insulin controlled, I mean not so severe — the hepatic glucose output is increased to raise the blood glucose level so that the peripheral utilization of carbohydrate can be improved In spite of this the peripheral utilization is still impaired (Figure 22)

As a last speculation we have tried to compare the hepatic and peripheral action of insulin in the various types of diabetes We have used the "insulin area" and the 'hepatic uptake" (Figure 23) I have already discussed the "hepatic uptake" The "insulin area" is the area bounded by the initial blood glucose level and the depression in blood glucose occurring after insulin Now, the hepatic area measures the liver's response to insulin in terms of hepatic glucose output The insulin area is an estimate of every single process by which insulin could lower blood glucose In other words, it measures hepatic removal, peripheral removal and urinary loss This is the only way we managed to get at the peripheral side of insulin action And in normal subjects these two correlate The greater the hepatic uptake after insulin the greater the insulin

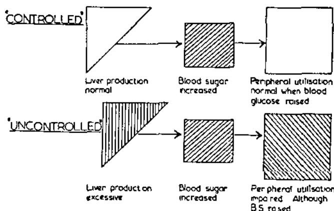


FIGURE 22 Hepatic glucose output and peripheral utilisation of carbohydrate in diabetes

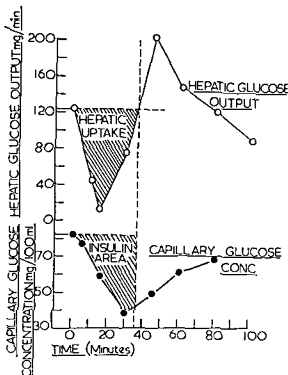


FIGURE 23 The hepatic uptake measures the diminution in hepatic glucose output in grammes and the insulin area measures the removal of glucose from the capillary blood in mg/minute for thirty minutes after intravenous insulin Reprinted by permission from Sherlock S Hepatic vein catheterisation in clinical research *Proc Inst Med (Chicago)* **18**, *335 (1951)*

area In other words, the effect of insulin in increasing peripheral utilization of glucose and in diminishing the hepatic output of glucose goes hand in hand This applies to normal subjects Diabetics behave differently *In diabetics the points describe a sigmoid* curve (Figure 24) Some diabetics have relatively better insulin areas than they do hepatic uptakes These are the obese ones with fatty livers I put it forward that these obese, insulin insensitive diabetics show a greater peripheral action with insulin than they do hepatic The hepatic sensitive group, usually young and thin, have a relatively greater hepatic uptake than they do insulin area The differences between the two types of diabetics is shown in Table I

Obviously this is too hard and fast a distinction and patients will be encountered who will fall between the two types and, in

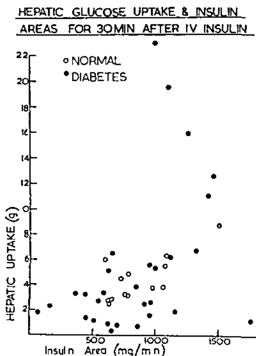


FIGURE 24 The relation between hepatic glucose uptake and insulin area for thirty minutes after intravenous insulin

particular an occasional elderly thin diabetic may show a hepatic insulin sensitive pattern

Stetten There are a couple of points I want to comment on

In discussing the early response to insulin mention was made of epinephrine response and mention was also made of the purported alpha cell hormone It is of interest I think that these two substances are as far as current evidence indicates operative at the same point in the glycogenolytic cycle According to the evidence of Sutherland(32) both epinephrine and the hypoglycemic glycogenolytic factors supposed to arise from alpha cells operate in the same fashion to reactivate phosphorylase which has become inactivated Why this effect should in all cases result in the disappearance of glycogen and never in the accumulation of glycogen is a riddle which so far as I am aware has not been solved

TABLE I

Summary of Results

	Hepatic Sensitive	Hepatic Insensitive
Age	Young	Old
Weight	Normal or Thin	Obese
Ketosis	Frequent	Never
Cardiovascular Complications	Rare	Frequent
Liver Biopsy Histology	Normal	Fatty
Fasting Hepatic Glucose Output	Normal Unless Pre coma	Normal
Glucose Uptake After Insulin		
Liver	+++ (unless ketotic)	Slight
Peripheral Tissues	++	+

The second point which occurred to me related to conversations with Dr George Guest(33) of Cincinnati, which I think perhaps contribute to the explanation of the effect that Dr Sherlock mentioned namely, the relative insensitivity to insulin in one subject in ketosis whose liver became more sensitive to insulin when he was out of ketosis

Dr Guest, as you may know, has maintained dogs in severe acidosis of nondiabetic origin, that is, by continuous ammonium chloride injection and finds that when the pH of the blood falls, the sensitivity to insulin diminishes The chemistry of this I am not able to explain, but the facts, I think, are quite convincing And when the pH of the blood is at 7 or below – and he has maintained it at such low levels – the dog becomes essentially completely insensitive to insulin

When Dr Best wrote to me suggesting that I comment on Dr Sherlock's paper, I felt some hesitancy in that my experience with diabetes was limited to rats I think, however, the results that we have been obtaining, do perhaps integrate better than what might be expected with the results she has presented

The experiments(34) I am going to talk about are a collaborative effort carried out partly in our laboratory in New York, and partly in the laboratory of Dr Dwight Ingle in Kalamazoo, Michigan

It is a nice collaboration in that what we do is very easy and what he does is very difficult, as demonstrated in Figure 25 *

Rats have been employed These rats are what he insists on calling normal rats but which I prefer to call previously untreated rats Alloxan diabetic and phlorhizinized rats have been compared The rats are maintained for several weeks prior to the experiment on tube feeding in order to insure so far as possible a constant intake of a medium carbohydrate diet They are fasted for 24 hours prior to the experiment They are anesthetized with a barbiturate, cyclopal, and a tracheal cannula is inserted which derives oxygen at atmospheric pressure from a partially inflated balloon and from which the CO_2 is quantitatively removed by recycling the expired gas through alkali A catheter is inserted into the bladder to permit rapid urine collection and a needle is introduced into the femoral vein, which is connected to Dr Ingle's constant infusion machine, whereby material can be infused at a constant and predetermined rate

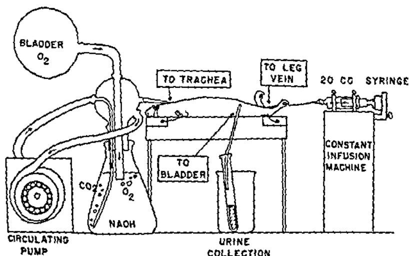


FIGURE 25

* Figures 25 through 31 and Tables II III IV and V reprinted by permission from Welt D Ingle D J Morley E H and Stetten DeW Jr The rates of glucose production and oxidation in normal and diabetic rats *J Biol Chem* (1951)

The material infused in these experiments was a solution of radioactive glucose, uniformly labeled, prepared by the photosynthetic method using bean leaves The infusion rate was adjusted so as to insure constant glucosuria In other words, the injection rate was relatively high, and high levels of blood sugar were thus obtained

If one does this in a normal rat, one obtains results which are seen in Figure 26 It will be seen that during the first few hours the specific activity of the urinary glucose rises rapidly and then, after the third hour, shows little tendency to further rise, attaining a plateau

In the normal rat, in order to insure glucosuria, we had to inject glucose at a relatively high rate

In Figure 27 are shown results with a phlorhizinized series of animals

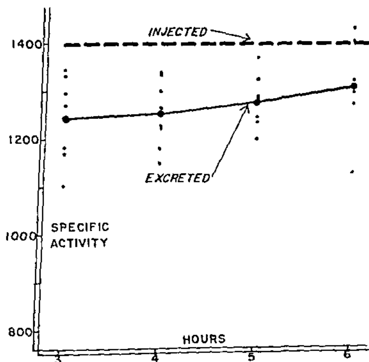


FIGURE 26 The specific activity of lucose in the normal rat

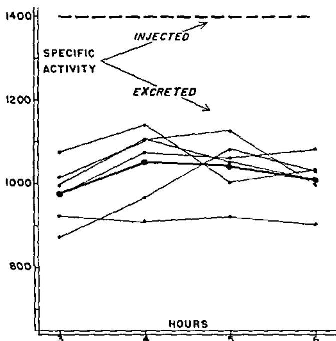


FIGURE 27 The specific activity of urinary glucose in phlorhizinized rats

Here because of lower renal threshold, we were able to inject glucose at a considerably lower rate, which is advantageous from the point of view of mathematics, as I shall show you in a moment

We then carried on similar experiments with alloxanized rats, which were moderately severely diabetic, excreting 8 grams of glucose per rat per day on the ration given Figure 28 shows the initial results

Here it is quite apparent that we were not, at the end of 6 hours, on a plateau The specific activity of the urinary glucose was still rising This we have attributed to the fact that the reserve of glucose in alloxan diabetic animals was probably a great deal higher than the reserve of glucose in the phlorhizinized animals, and consequently a more prolonged period of washing out was necessary before a uniform level of isotope concentration was found in the excreted glucose Experiments were therefore carried on for 12 hours, as shown in Figure 29 Here may be seen the data presented in Figure 28 together with longer experiments, and in the

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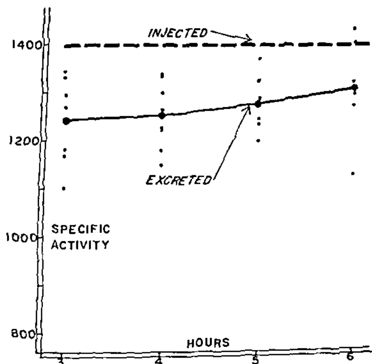


FIGURE 26 The specific activity of urinary glucose in the normal rat

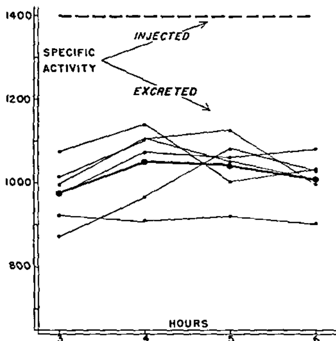


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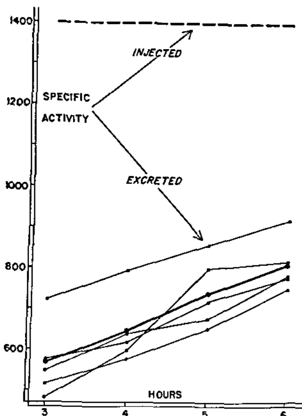


FIGURE 28 The specific activity of urinary glucose in alloxanized rats

portions of the 12 hour period we felt that a reasonably good plateau had been obtained and the experiments were therefore terminated

Figure 30 shows the nature of the arithmetical process When the abundance of isotope in urinary glucose shall have come to a steady value then from a comparison of the specific activity of the injected glucose and the specific activity of the excreted glucose if one knows the rate of injection one may compute another rate *This rate which we have designated by "r is the rate at which* the tissues of the animal are producing glucose from nonisotopic precursors It excludes such glucose as the rat may form from products that are themselves derived from glucose during the experimental period

By taking the data which you have just seen we have computed this rate in the three situations described (cf Table II)

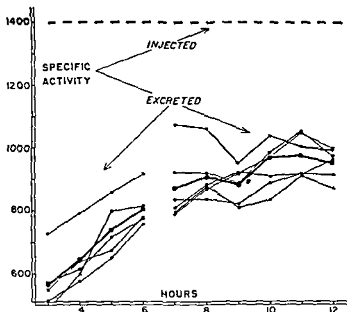


FIGURE 29 The specific activity of urinary glucose in alloxanized rats

In the untreated rat, some 34 mg of glucose per hour were arising from non isotopic precursors under the conditions of this experiment Phlorhizinization had no effect on this figure, which probably also indicates that the rate of glucose production in this type of experiment is relatively independent of glucose load because in the phlorhizinized animals one quarter as much glucose was being injected per hour as in the untreated animals

WHEN A STEADY STATE IS ACHIEVED

IF A = specific activity of injected glucose (cts/min/milliatom)
R = rate of injection (mg/hour)
a = specific activity of excreted glucose
r = rate of "gluconeogenesis"

THEN

$$AR = a(R + r)$$
$$r = R(A/a - 1)$$

FIGURE 30 Nature of arithmetical process (see text)

TABLE II

Rate of Glucose Formation

	a	r
	c/m/milliatom	mg/hr
*Untreated	1271 ± 15	33.8 ± 4 3
**Phlorhizin	1020 ± 16	31 0 ± 1 8
**Alloxan	921 ± 13	43 3 ± 1 8

A = 1400 c/m/milliatom
*R = 33 mg/hr
**R = 83 3 mg/hr

In the alloxan diabetic animals there is apparently a small increase over normal in the rate of glucose production The increase over normal is barely statistically significant The difference is certainly small *amounting to some 10 mg per rat per hour* This figure may be compared with the 200 300 mg which these rats are hourly excreting in their urines It is quite apparent that this increase is making a very minor contribution if any to the glucosuria which is observed in these animals

TABLE III

Glycogen From Glucose

	Per cent
Untreated	81
Phlorhizin	28
Alloxan	—

In Table III are shown the results from the glycogen in the livers of these animals at autopsy The alloxan diabetic animals showed no demonstrable glycogen in their livers at all On the other hand from the untreated and phlorhizinized glycogen could be obtained relatively less from the phlorhizinized animals

Comparing the specific activity of the glycogen in the liver with that which was present in the circulating blood during the last 4 hours of the experiment it can be shown that at least — and this

is a minimal figure — that at least 81 per cent of the glycogen present in the liver had arisen in the normal animals from circulating glucose The corresponding figure in the phlorhizinized animals was 28 per cent This difference we do not entirely understand but is perhaps a result of the lowering of the renal threshold and consequent lower blood glucose level in the phlorhizinized group of animals

The study of the exhaled CO_2 proved to be rather complex The reason is shown in Figure 31

Here is one rat which was carefully studied in which the excreted glucose obtains a reasonable plateau after three or four hours but it will be observed that the expired CO_2 is rising continuously in radioactivity The curve appears to be biphasic, made up of a fairly rapid component and a slower component, but the significance of that I would rather not go into Certainly after 12 hours there is no indication that this rise seems to be stopping

We construe this to mean that with the passage of time, more and more devious pathways by which carbon initially present in glucose can be delivered to CO_2 are being included in our method

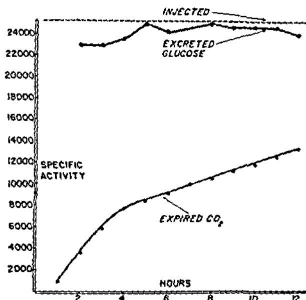


FIGURE 31 The specific activity of urinary glucose and expired CO_2 during intravenous administration of C^{14} glucose to a normal rat

of measuring And it is impossible, I think from this type of experiment to state which pathways are *included and which pathways* are excluded One may anticipate that this value will continue to rise as a function of time until every carbon atom in the body of the rat which is derivable from glucose shall have attained a constant specific activity, and this may take a very long time indeed

In order to get some information out of our experiments, therefore, we became arbitrary, and we decided to compare the CO_2 with the blood glucose with respect to specific activity at that time when the blood glucose had attained a plateau value, and the results obtained are shown in Table IV

TABLE IV

Rate of CO_2 Production From Glucose

	Per cent	ml /hr
Untreated	22 6	0 90
Phlorhizin	7 0	0 19
Alloxan	9 4	0 31

I should like to stress that this is an arbitrary judgment and that if we had selected a later period of time, then all of these figures would have been larger But selecting that period of time, it appears that some 22 per cent of the CO_2 exhaled could be accounted for as arising from blood glucose in the untreated animals We were rather surprised at how small that figure was *I think it is generally* felt that if you start a glucose infusion and maintain it for several hours at a level such as that glucosuria occurs probably the animal *is* burning chiefly glucose This, apparently is not the case

In the phlorhizinized animal the corresponding figure was perhaps a third as high And, here again, contributing to this decrease *very likely, is the somewhat* lower levels of blood sugar in these animals

In the alloxanized animals such an explanation, however, does *not obtain, because these animals certainly* had high levels of circulating blood glucose

The total results to date are shown in Table V, where we have *recalculated the data on the basis of* 100 g of rat tissue, and it

TABLE V

Mg Glucose/100 gm. rat/hour

	Untreated	Phlor	Allox
Formed	15 0	14 0	21 0
Burned to CO_2	11 4	2 7	4 5

will be observed, as was mentioned before, that the phlorhizinized and untreated rats are generating glucose from nonisotopic precursors at the same rate, the alloxanized perhaps a little faster But the rates of glucose oxidation are lower both in the phlorhizinized and in the alloxanized animals than they are in the normal

These results fit in quite well I believe, with recent results from the laboratory in LaJolla of MacKay and collaborators(35) They are in contrast to results published from Chaikoff's laboratory(36), and I would rather not discuss my explanation for the difference unless that is brought up in discussion It is a technical matter

This decrease in the capacity of the alloxan diabetic rat to burn glucose to CO_2 is, I believe, in accord with other observations of our own and of other laboratories that many of the fats of glucose can be shown to be inhibited in the alloxan diabetic animal This includes, among other things, the capacity of the diabetic animal to generate fatty acids on a high carbohydrate diet, which is essentially completely inhibited in the severely diabetic rat(37), and has been shown now in the isolated liver(38)

Artom Dr Stetten, I should like to hear your comment about the similarity between the phlorhizinized rats and the alloxanized rats How do you interpret that?

Stetten Well, there is a phrase of Dr Best's that I have always liked, and that is "a diabetic rat is starving in the midst of plenty" I think the phlorhizinized rat is just starving

Will that do Dr Best?

Best That is fine

I should like to see another series of completely depancreatized rats

Stetten We hope to do that among other things

Hartroft Had the alloxan produced any damage in the livers of the animals?

Stetten I cannot say for sure Those livers get popped into alcohol very fast at the end of the experiment We could take biopsies which had not occurred to us

Hartroft When was the alloxan given?

Stetten The alloxan was given in all cases 3 weeks prior to the experiment

Shorr May I ask the definition of overproduction? With respect to what?

Stetten With respect to the previously untreated rat you mean what precursor this type of experiment gives no indication of what the precursor of the newly formed glucose is

Shorr Did you do nitrogens in the urines?

Stetten We did not do nitrogens

Shorr I think that in the statement of the overproduction theory presented by Dr Sherlock many of the points of the theory advocated by Soskin are not expressed in that the source of the glucose is postulated to be fat isn t that true?

Sherlock Yes

Shorr So in dealing with the entire concept approaching it through the methods that Dr Stetten has used one must bear in mind the nature of the precursor

Sherlock I think I should also say in all fairness that our observations on the initial output of glucose from the liver in diabetes have been based on results for a limited period of observation that is 3 blood samples have been taken over periods of 40 minutes It seems important to measure the findings over 12 hours and I would like to do that and with much more careful urine analyses (Table VI)

TABLE VI

Fasting Hepatic Output of Glucose in Normal and Diabetic Subjects

	No	Capillary Glucose Concentration (mg/100ml)	Fasting Hepatic Glucose Output mg/Sq m/min
Normals	43	82 6 ± 1 2	116 ± 9 4
Diabetics	39	233 9 ± 20 5	129 ± 14 4

Shorr All throughout your biopsies showed plenty of glycogen This could be the source of the glucose In other words you did not have to invoke the production of glucose from fat or from protein in your diabetics?

Sherlock No

Shorr So that you might consider using increased release of glucose" rather than "formation which involves the source of the glucose

Sherlock It is of interest though that diabetics, on the whole have perhaps little more glycogen in their livers than normal subjects And I would say that if the overproduction does occur it seems unlikely that it is occurring from glycogen I agree with Soskin that it is probably occurring from fatty acids

Shorr On what evidence?

Sherlock Well, there is an awful lot there if they are over producing

Shorr Yes but you have not any direct evidence in the human for that

Sherlock No

Best Surely Dr Soskin(39) would not have said that all the production of glucose was from fat He would say that it was from protein and also fat Many of us dont follow him I never have Although he was my first Ph D student I have never followed his conclusion that there was evidence for the production from fat but I think that he would not discount the importance of glycogenesis from protein

Shorr And with respect to the peripheral utilization I would take it Dr Stetten that your own studies would show in the phlorhizinized *animals that are receiving glucose* that the blood sugar levels were low all throughout the period of phlorhizination?

Stetten Probably lower than the normal

Shorr You dont know?

Stetten Initially they were normal but we wanted to distinguish the rats in the course of the experiments

Shorr One of the well established facts about phlorhizin diabetes is that as you continue to feed sufficient glucose to a phlorhizinized

animal, it progressively resumes a normal capacity to oxidize glucose

Stetten That is true I can say this, Dr Shorr that the phlorhizinized animal continues to have glycosuria at a load of 83 mg per hour

Shorr Oh, he will continue to have glycosuria?

Stetten Yes whereas the normal rat requires at least three, and probably four, times as much glucose in order to have glycosuria

Shorr Yes, but in spite of the persistence of glycosuria, he regains his ability to oxidize carbohydrates with time

Stetten Yes

Shorr I don t know how soon the rat regains this capacity If in the course of your 12 hour experiments there should be such a shift in your phlorhizinized animal, then I think it would be important to make sure that it does not arise from the enhancement of the blood sugar level up to that which at least in other animals, causes a resumption of carbohydrate oxidation

Best I was wondering in Dr Sherlock's two types of diabetics, just how much of that difference is due to the difference in the amount of insulin which those two groups have available

Sherlock Well, it could be, and the work you quoted earlier would support that I mean the amount in the pancreas

Best I mean if you have a certain amount of insulin available and you add more, it is going to have less effect than if you added it to a vacuum, for instance

Sherlock Yes, indeed But I find it difficult to believe that all the clinical differences between the two could be just on that basis. I mean they are so different There are so many differences Their complications — everything is so different about them

Stetten Is there any correlation between what you have classified as the hepatic sensitive group of diabetics and sensitivity to insulin?

Sherlock General sensitivity to insulin?

Stetten Yes

Sherlock Oh, yes

Turner I did not get quite clearly, Dr Sherlock, how much the difference between the normals and diabetics could be accounted

for by the difference in epinephrine release It seemed to me that a great deal of it could be explained on the difference between starting with a high blood sugar and not eliciting a response of hypoglycemia

Sherlock I think we took care of that by only taking our observations for 30 minutes before the epinephrine is released If one compared the differences between normals and diabetics over two hours I think that one would have radical differences based on epinephrine release in one and not in the other But if one stops short at 30 minutes then I think the two are comparable

Turner Can you be sure that the epinephrine effect does not begin sooner than that or could it be that the difference in balance between the two merely becomes apparent after that length of time?

Sherlock We did not find other evidences of adrenalin release before 30 minutes after the insulin was given to the normal subject Not only is there no increase in hepatic blood flow but there is no increase in blood lactic acid level or in splanchnic oxygen consumption All these things occur very rapidly after the administration of adrenalin

Watson There is one point about the fatty liver that perplexes me I imagine there is a ready explanation for it that has not occurred to me I have repeatedly seen cases in the past especially among children who were what one would have to classify as labile diabetics in the sense that they had insulin reactions quite easily and yet developed ketosis and had very fatty livers extremely fatty livers Now it is true that they probably developed the fatty livers subsequent to the ketosis but one wonders from your data whether the fatty liver is supposed to protect against ketosis and to make the individual insensitive to insulin At least in these individuals – and I should like to hear what Dr Gyorgy thinks about it – it seems to me that this is more particularly seen in children the combination of insulin insensitivity and tendency to ketosis in the presence of a fatty liver

Sherlock Well for instance Dr White(40) long ago referred to hepatomegaly in juvenile diabetics and suggested that the hepatomegaly was due to fatty liver Now if you read the literature on biopsy evidence supporting that it is practically nonexistent And most of these associations of fatty liver and ketosis in young subjects have been based on autopsy evidence and the cause of

the fatty liver is usually quite evident in terms of recurrent pneumonia or gastroenteritis, or something of that nature I believe that the hepatomegaly of the young diabetic is probably due to increased glycogen content and water in the liver, but I have done few biopsies on children, so I could not prove it

Watson We have some biopsies

I am thinking of one patient in particular who was a young woman who presented exactly the combination I mentioned in which there was a rather fatty liver I would say at least a 3 plus on the basis of your classification

Sherlock And she suffered from nothing else apart from diabetes?

Watson Nothing else that we could find

Sherlock And she was not overweight?

Watson No she was not I would say she was approximately normal in weight

I am also thinking of a young girl of 13 who died of coma but who had been, prior to the onset of ketosis and coma, a very labile diabetic, sensitive to insulin At autopsy she had an extremely greasy yellow liver, just loaded with fat

Gyorgy We don't have any biopsy findings Therefore I couldn't argue either in favor or against Dr Sherlock I think in autopsies we see fatty liver, but unfortunately we don't know when this fatty liver occurred

Watson Yes

Gyorgy However, with regard to fatty liver, I should like to state an old problem You have old diabetics with fatty liver You may assume, and probably you will assume, that this fatty liver is of long duration, because they probably had had their diabetes for a long time Why don't you see cirrhosis more often in the patient with long standing fatty liver if fatty liver is the first stage to cirrhosis? Or is there any of the pathologists here who could distinguish this type of fatty liver from the fatty liver which will lead to fibrosis and what we call cirrhosis?

Sherlock I think you are in a better position to answer that than I am I don't think we all believe that fatty livers and cirrhosis are carts and horses?

Gyorgy I don't

Sherlock Well, this evidence would suggest that is not so

Gyorgy I thank you

Watson Might it not be a matter of the duration? You haven't really any evidence as to the duration of the fatty liver in those people you talked about May it not be a matter of how long they have a fatty liver continuously in relation to whether cirrhosis develops or not?

Sherlock But I think Dr Gyorgy is right These patients are perhaps 70 years old and their diabetes started at 50, and they have been overweight for that length of time and yet they don't have any evidence of fibrosis, although they have fatty livers

Best I think one thing I will make clear is that I feel that the cirrhosis resulting from choline deficiency is preceded by a fatty liver

Gyorgy I do, too

Best Definitely

Gyorgy I don't know if it is a prerequisite It could be coincidental

Best I just said it is preceded by a fatty liver

Gyorgy Yes

Hill Dr Sherlock, didn't you say that one of your group was not obese?

Sherlock Quite a number of those diabetics with relatively fatty livers were not frankly obese

Hill Have you any simple explanation? My ideas of this are very, very elementary, I am sorry to say Have you any explanation for that sort of thing?

Sherlock Well, the only suggestion is the one I put forward That is that the pituitary does have a fat mobilization factor which acts either directly or by some liaison with the adrenal cortex, and produces mobilization of fat from depots and increased fat in the liver, and this factor has been identified by some with the growth hormone and also with the diabetogenic hormone of the pituitary It would link up the diabetes and the fatty liver, but it is pure speculation

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Sherlock But I think Dr Gyorgy is right These patients are perhaps 70 years old and their diabetes started at 50, and they have been overweight for that length of time, and yet they don't have any evidence of fibrosis, although they have fatty livers

Best I think one thing I will make clear is that I feel that the cirrhosis resulting from choline deficiency is preceded by a fatty liver

Gyorgy I do too

Best Definitely

Gyorgy I don t know if it is a prerequisite It could be co incidental

Best I just said it is preceded by a fatty liver

Gyorgy Yes

Hill Dr Sherlock, didn t you say that one of your group was not obese?

Sherlock Quite a number of those diabetics with relatively fatty livers were not frankly obese

Hill Have you any simple explanation? My ideas of this are very, very elementary, I am sorry to say Have you any explanation for that sort of thing?

Sherlock Well, the only suggestion is the one I put forward *That is that the pituitary does have a fat mobilization factor which* acts either directly or by some liaison with the adrenal cortex, and produces mobilization of fat from depots and increased fat in the liver, and this factor has been identified by some with the growth hormone and also with the diabetogenic hormone of the pituitary It would link up the diabetes and the fatty liver, but it is pure spec

Hartroft I would like to comment on the problem of the etiological relation of abnormal amounts of liver fat to the development of hepatic fibrosis Although all types of cirrhosis are not preceded by fatty change, and although conversely, fibrosis may not be present in all fatty livers, it is our opinion that when fibrosis does develop in fatty livers this is the direct effect of the rapid accumulation of lipid to an excessive degree in the affected regions Fibrosis is simply a morphological indication that the hepatic parenchyma has been damaged in an irreversible way It is quite possible for the liver to store appreciable amounts of fat without destruction of parenchyma Irreversible damage to liver cells does not occur if the amount of lipid present is not so great that it can still be stored in intracellular form This type of fat storage in the liver without fibrosis was achieved in an experiment reported by Handler and Dubin(41) Their rats were fed a diet containing 18 per cent casein with a supplement of nicotinamide The fat content of the livers of animals killed after 8 weeks had reached approximately 22 per cent and *remained* at this level throughout the course of the experiment (120 days) at which time only slight fibrosis was present in a few rats From these and other experiments, the authors suggested that the hepatic fibrosis of choline deficiency may be the result of chronic fatty infiltration and that the ingestion of an adequate quantity of good protein protects the liver from the deleterious effects of chronic fatty infiltration but this protective capacity is not based on lipotropic activity alone We suggest that the absence of fibrosis in their experiment can be explained by the fact that after the fat content of the animals' livers rose to 20 per cent the first 8 weeks it *did not increase* thereafter until the last group of rats was sacrificed at the end of 120 days It is suggested here that the absence of appreciable amounts of fibrosis in these fatty livers was not due to some unknown protective action of the dietary protein but to the fact that the accumulation of fat increased neither to a sufficient degree nor with enough rapidity to distend the liver cells to the point where they would burst and form the fatty cysts which we have described at previous conferences of this Foundation Detailed histological investigations of the pathogenesis of the fatty type of cirrhosis which develops in the livers of rats fed diets low in choline have convinced us that the fatty cysts (or lipodiastaemata) are the essential metaplastic link between the fatty and fibrotic liver The formation and subsequent atrophy of fatty cysts with their replacement by reticular condensation in the form of fibrous trabeculae does not occur unless the accumulation of fat is

sufficiently rapid and great (at least in localized areas which are usually centrolobar) that the capacity for intracellular lipid storage by individual liver cells is exceeded On this basis it is almost axiomatic that the excess accumulation of fat in the liver is not only the forerunner but also the direct etiological agent responsible for the fibrosis Only under such circumstances does fatty change in the liver produce fibrosis and the mere presence of stainable fat *per se* is not sufficient to induce irreversible parenchymal damage In the human cases you are discussing the fatty change may not have been sufficiently great to fulfill the conditions leading to formation and rupture of fatty cysts with subsequent fibrosis produced by associated reticular collapse Such findings do not conflict with the hypothesis that the type of hepatic cirrhosis associated with a dietary deficiency of lipotropic factors is caused by the accumulation of excess amounts of fat in ever increasing amounts It is largely a matter of the amount of fat present in the liver and its distribution within the organ Fat which is largely concentrated in restricted lobular areas will be more damaging than the same amount distributed more uniformly throughout the entire parenchyma so that each cell need store a smaller share of the total Distribution may be affected by many factors such as the rate at which the lipid accumulates etc My colleague Professor E A Sellers has recently made some observations concerning the influence of the thyroid on the distribution of fat within the liver lobule in choline deficiency and will be publishing this material in the near future

Watson Mr Chairman I might point out for the record that there have been at least two papers in the last two years citing a considerable number of cases of cirrhosis and fatty liver in diabetics I am unable to cite the references at the moment

Gyorgy I know the paper of Remberg and Lapson(42) The author's conclusion is that "there is no significant difference between the incidence of Laennec's cirrhosis associated with diabetes mellitus and the incidence of cirrhosis in non diabetics"

Sborov Zimmerman and co workers(43) have a recent paper on this subject

Patek There have been other series that have denied that conclusion It is one of those problems in clinical evaluation Dr Best that are so difficult

Best I should like to have someone investigate the incidence of cirrhosis in diabetics before insulin In Dr Joslin's book(44) I kn

it is referred to and also cirrhosis in pernicious anemia Both these conditions produce fatty livers But you don t see the same types of cases today

I had assumed that in the acute cases of diabetes they would not live long enough to get cirrhosis but in a mild case with a little insulin available they might But it is a little difficult to go back now and find out what was the situation

Dr Sherlock in the first slide (Figure 7) you put on there was a reference to von Mering and Minkowski(45) They pointed out the large yellow fatty liver in diabetes and they were the first to do that A few weeks ago Frau Minkowski described those experiments to me Dr Houssay and I went to visit her in Buenos Aires She told us of her husband s trials and tribulations because he was the most active investigator in this particular study and she remembered that he had commented on fatty livers He apparently had talked these things over with her

Incidentally she presented me with a book of Minkowski s John Konrad von Bruner s book written a century ago and Rollo s book who was the first clinician to use dietary measures in the treatment of diabetes and also with a death mask of her husband My problems in getting these articles and others home were solved by a member of the United States Air Force and a member of the Canadian Air Force who had a plane between them and who took all the luggage including the books and the mask and flew them to Ottawa and thence to Toronto

Gyorgy Dr Best they were depancreatized dogs and the fatty liver could have been due to the absence of pancreas

Best It is not that kind of fatty liver I mean it is not due to a dietary lack

Gyorgy I mean what Minkowski had was this fatty liver in depancreatized dogs

Best The dogs did not live very long

Artom If I remember correctly the Minkowski s dogs did not live more than 2 weeks – at the most

Gyorgy In 2 weeks you get fatty liver

Best It is completely reversed by giving insulin The liver shrinks down to normal size and the fat completely disappears The action of insulin is more dramatic than choline

Hartroft How was the fatty liver produced in the experiments to which you refer?

Best By pancreatectomy Within 24 hours the liver returns immediately to normal(46)

Kinsely In relation to determination of fat in the liver histologically there is a new technique which is not being exploited fast enough according to my thinking Dr Adamstone at the University of Illinois Department of Zoology has developed a new technique for freezing tissues including liver and then cutting sections very fast and keeping them cold – and they never warm up – and getting them on slides(47 a b) When I visited his laboratory he had such specimens and tissues which had never warmed up showed many fine drops But when he allowed them to warm up – now I am trusting to a feeble memory – the things coalesced into freezing droplets

Hartroft Is that a freezing drying technique similar to that of Altman and Gersh?

Kinsely No drying

He has worked a long time you have to see him carry out the process to see how beautifully it works

Also Dr Arthur Woerner at the University of Louisville has been working on fat distributions testing various techniques and I think that he is coming toward the idea that lipoids change so rapidly that instantaneous preservation is important

Sborov Dr Best I should like to ask Dr Sherlock whether she feels the fatty liver in her group came first or whether the diabetes came first The reason I ask is that we have a number of patients now that we have studied somewhere between 6 and 10 some with cirrhosis and some without some obese and some lean who have had fatty livers that we have treated with choline and a high protein diet These patients had abnormal carbohydrate tolerance and a mild diabetes prior to treatment When the fat left their livers they then had normal carbohydrate tolerances

Sherlock I would say if I had to choose that the fatty liver came first because it is well known that obese subjects develop diabetes and I am sure that some of our obese subjects with fatty livers will develop diabetes

What I should like to stress is that the two are not cause and effect that the fatty liver is not causing the insulin insensitivity

Watson Dr Sherlock, there is one point I should like to ask about, do you feel that obesity regularly produces a fatty liver?

Sherlock I think overweight subjects, over 120 per cent, of their ideal body weight, will show fat in the liver

Best That is a challenging statement

Watson It surely is

Turner Is there a reference to that?

Best I don't know of any evidence to support that in animals but that is a different proposition

Hill Dr Sherlock, in your group of the insulin hypersensitive ones – they were a young age group?

Sherlock Yes

Hill Anything peculiar about them? Any oddities that you found out? Any high blood pressure?

Sherlock Oh, no

Fremont Smith Dr Best, are there any data on animals which clearly contradict this challenging statement that Dr Sherlock made?

Best No What I should like to see is insulin given to those patients to see if it would mobilize the fat from their livers

Fremont Smith But you made the statement that there was no evidence in animals to support that, and therefore I wanted to turn it around in terms of Dr Kniselv's talk on negative evidence Is there any possible contradictory evidence?

Best The evidence that I had in mind is this animals on a well balanced diet, even though they are obese, don't have fatty livers

Fremont Smith 120 per cent or over?

Sherlock Workers at Yale have shown that rats made obese by hypothalamic lesions show fatty livers(48)

Best That is a different proposition

Fremont Smith You know, in human obesity there is also forced feeding regularly from inner impulses, which is something like hypothalamic Generally I think that is a valid comparison

Watson Might I hear from some of the others? I had the impression when I was doing autopsies that I used to be amazed by the lack of fat in the liver in obese subjects Maybe I am entirely wrong about that I should like to hear the pathologists' comments on that

Campbell I would support the statement that one sees little fatty change They may have a little fat, but it is not something that strikes the pathologist in such manner that he says "Here is a good fatty liver"

Fremont Smith Has it something to do with causes of death?

Campbell They would be varied

Fremont Smith Would the terminal illness affect that?

Campbell I have no data about the breakdown into different types of terminal illness

Madden The duration of obesity, the rapidity of its development, and the terminal illnesses are so varied that I think the pathologist's opinion at the autopsy table would not be worth a great deal

Fremont Smith But obese accident cases would be very good, wouldn't they?

Sherlock Dr Ralli described fatty liver in overweight people at autopsy after sudden deaths(49)

Best I think what we are trying to get at is whether the obese patients who have fat in the liver are restricted to those who are pre diabetics or whether this is a universal finding in all obese people — excess fat in the liver

Sherlock Yes, I think it is a universal finding

Fremont-Smith In other words you would say that the pre diabetics are among those?

Sherlock Yes

Fremont Smith That they do not all constitute pre diabetics?

Sherlock Yes, indeed

[illegible]

without cirrhosis was almost the rule in absence of diabetes

Gyorgy Long standing fatty liver?

Smetana Fatty liver of long standing Over a period of years these people consumed from 10 to 12 liters of beer per day

Watson Now, Dr Smetana, if you are talking about the typical Munchener who used to drink ten to twenty liters of beer, my own observation of those individuals was that their diets were not very good that some of them lived on beer very largely

Gyorgy Oh, no

Fremont Smith You only watched them when they were drinking You did not watch them when they were eating

Smetana They ate very well

Watson You are sure you got the ones that ate well

Knisely Gentlemen, there is a question of techniques Most of these determinations of fat seem to boil down to histology so far, and Dr Turner presented a beautiful paper of selection of things in the general fat world, lipids Would it be possible, among the pathologists, to take a specimen, either biopsy or gross autopsy, macerate it, run it through a chopping machine, centrifugate it, and find out how much separable liver fat there is, or has this already been done? I don't know that world

Hartroft We have correlated our histological observations on the livers of choline-deficient rats with quantitative biochemical determinations of the amount of lipid present in the same livers performed by Professor C C Lucas and Doctor Jessie H Ridout in the Banting and Best Department of Medical Research at the University of Toronto Quantitative estimations on a histological basis expressed as 1, 2, 3 or 4 plus have agreed very well with the biochemical determinations

Artom However, it is my impression that biochemists who are working on experimental animals do not talk of fatty liver until there is a considerable increase in the amount of fat, whereas, pathologists sometimes call a liver fatty as soon as they see on their slides some fat in the liver cells If chemical determinations were made, little or no significant increase of fat above the normal level would be found in such livers

Knisely Are these the same fats, or a series of different fats? I was wondering whether in autopsies if one took people with diabetes a short time, and did liver fat determinations and people

who have had it a longer time would one begin to see changes in the fat distribution changes in the fat distribution as well as in the quantity Is that worth doing?

Turner Oh yes It seems to me we are in need of more differentiation

Knisely I think we are wasting autopsy material What can we learn from autopsies that we are throwing away?

Sherlock Not much From biopsy material yes from autopsy material no

Knisely Oh! I am going to disagree and in the same breath hope to maintain your friendship It seems to me that there are many kinds of information which could be obtained from autopsy material if that material were studied by each one of many different kinds of analytical methods

For instance we are not doing today very much biochemical analyses of materials from autopsies We now know a great deal about the chemical metabolism of bacteria and we know a great deal about proteins which may be obtained from human blood Chemical analyses of autopsy material taken immediately after death might contribute to an understanding of how organisms are altering the chemistry of blood plasma Professor William Welch in his section on thrombosis(50) lists a great many pathological organisms which had been found in large thrombi And since that time we have learned factors of the metabolism of bacteria which might effect blood coagulation and since then antibiotic drugs have been introduced into the practice of medicine some of which have been thought to be able to alter or affect processes of blood coagulation Also pleomorphic forms of organisms are now known which Welch did not know Thus I feel strongly that both biochemical and bacteriological examination of large thrombi should be reinitiated And I strongly suspect that we are losing many other possible kinds of information by not paying the right kind of attention to autopsy material I beg to differ with you

Best Other questions?

Neefe I should like to make one comment on the question Dr Knisely raised in respect to fatty livers It seems to me that very frequently at least in human liver biopsy material you see two distinctly different types of fatty liver There is the one with the gross large vacuoles that are so common and another type which

often is apparent on an H and E section but shows large numbers of small fat droplets in the cell with special fat stains Thus, if one classifies the liver on the basis of the H and E section's appearance, one might very readily falsely classify the liver as nonfatty I should like to hear what the pathologists would say about this One wonders, too, whether there are the same types of fatty livers or whether we may not have different kinds with different clinical significance

Popper In answer to Dr Neefe's question, I believe we all agree that for a reliable interpretation as to the presence or absence of fat, special stains like the Sudan stain are necessary I discussed this question often with Dr Smetana and I believe also, with Dr Hartroft We all agree that vacuolization in the hematoxylin-eosin section is misleading

To come back once more to the problem of the relationship between overeating and fatty liver, my impression agrees with what Dr Sherlock and Dr Smetana have said, however, I would say it is not consistently so I would like to emphasize that one should differentiate between fatty metamorphosis in obesity and central fatty changes which, for instance occur in anemia The latter are characterized by small fat droplets in the centrolobular zone I am sure some of the latter changes develop in the pre mortal period

Many years ago I tried to do something which Dr Knisely has just suggested namely, to identify fat a little better by histologic methods I was studying the fluorescence microscopic distribution of vitamin A Vitamin A is a green fluorescent substance in the liver much of it is dissolved in the fat droplets producing so to speak a vital staining This is only missing in marked vitamin A deficiency which is rare in our part of the world The fat droplets we see give rather consistently vitamin A fluorescence Alterations in quality and quantity of this fluorescence characterizes fat histologically(51) I would like to mention here that I believe that the large fatty cysts in cirrhotic livers which Dr Hartroft described(52) show, as a rule little vitamin A fluorescence I remember having seen that and I still feel that I was very ignorant not to have realized their significance at that time I also remember some cases in which obesity appeared to be the only explanation for a high vitamin A concentration in the liver This may also support what has been said here

Best Dr Hartroft, did you have a comment on this subject of fat infiltration of the liver in diabetes?

Hartroft Perhaps we might make brief reference sir to a dog in our laboratory which had suffered spontaneous diabetes sections from the liver of which I showed you just recently The centro lobular regions were characterized by excessive fat storage with fatty cyst formation and here there were evidences of early fibrosis

Sherlock Was the pancreas normal in terms of acinar tissue?

Hartroft Yes The acinar tissue was normal but the pancreas was almost devoid of islets In a total of 30 to 40 sections taken from three blocks of tissue representing head body and tail of the organ not a single islet could be identified An assay of the insulin con tent of this pancreas proved to be extremely low

Best Yes very low

Popper Glycogen in the liver?

Hartroft There was little glycogen in the liver cells which were distended instead by fat

Shorr What was the preceding history of that animal?

Hartroft The dog was a spontaneous diabetic which was dis covered by accident by its owner and brought to the laboratory

Shorr How did it get along? Did it require any treatment?

Best We have had several We brought them in and balanced them on insulin and sent them home

Watson How are they diagnosed? What do you notice?

Best By the clinical signs

Stetten All dogs have polyuria

Watson It is relative

Stetten I was discussing with Dr Sherlock a matter discussed at the recent conference in Indianapolis Growth hormone has been posed as Dr Sherlock mentioned as one of the agents in the pituitary which apparently does increase the quantity of liver fat There was a report by Dr deBodo(53) in Indianapolis to the effect that in the hypophysectomized dog if I recall correctly the insulin sensitivity can be completely or almost completely elimin ated by the administration of small amounts of growth hormone And Dr Sherlock assured me that was in effect in the right direction

Best That would be an effect on the periphery rather than the pancreas you mean?

Sherlock No that the fatty livers were the ones that were hepatic insensitive in which you might invoke growth hormone increase although there is no collateral evidence that growth hormone is increased in these people

Gyorgy Or at least increased pituitary function

Sherlock Yes increased pituitary function" is better

Artom Many years ago we did a rather large number of analyses on the fatty livers of depancreatized dogs(54) It was our definite impression that the fatty infiltration was much more massive and much more easily produced in very young animals I remember one case of a puppy which after removal of the pancreas had only traces of glucose in the urine When we killed the animal we found that we had left some pieces of pancreas attached to the duodenum However in the liver there was a tremendous degree of fatty infiltration So the age of the animal has something to do with the fatty infiltration of the liver at least in depancreatized dogs

Best Well it certainly has in the rats When they are rapidly growing ones they have a much greater demand for the lipotropic factors

Artom You can interpret that both ways of course

Best Yes

Artom The case I mentioned seemed to me interesting also because in this puppy there was little disturbance in the metabolism of carbohydrates whereas obviously the metabolism of fat was altered to a very high degree

Best I was talking to Dr Hanger earlier today and to my mind there is a hypoinsulin fatty liver and hypolipotropic fatty liver but perhaps unfortunately you can influence the one due to lack of insulin by giving large doses of choline and probably you can influence slightly the one due to lack of choline by giving large doses of insulin So it is not quite a clear picture However I think that with reasonable doses of either one or the other there is not any effect on the fatty liver due to the other cause

Sherlock The evidence in the literature about the treatment of diabetic patients who have fatty livers with choline is very con

flicting We tried choline on three diabetics with fatty livers without any success in removing the fat

Best In the cases of hepatomegaly that Dr Priscilla White described they certainly tried choline and got nothing When they changed from regular insulin to protamine insulin the hepatomegaly disappeared in all of the cases

Sherlock I think it indicates that the diabetes was better controlled

Best That is what I think

Were the hepatic insensitive diabetics influenced by the diet when on a high fat diet Dr Sherlock?

Sherlock I had them only on a high carbohydrate diet

Turner Dr Sherlock is there a lipid difference in this insulin effect?

Sherlock I m afraid I m not able to answer that I have not estimated the serum lipids

Watson Dr Best I should like to mention briefly an observation that we have made recently that I think is interesting with respect to Dr Sherlock s group of insulin refractory older obese individuals This was a woman with a severe diabetes very refractory to insulin almost totally insulin insensitive She did not ever have an insulin reaction but her glycosuria was very difficult to control She had a severe peripheral neuropathy Pain was the principal complaint She was malnourished She had moderate hypertension and some hirsutism But otherwise in no sense did she have any suggestion of a true Cushing s syndrome Nevertheless her urinary 17 ketosteroids were found to be 22 mg per day and she was consequently given intensive X ray therapy to the pituitary She was then followed for a little less than three month The last time we saw her her ketosteroid excretion had declined to 7 her insulin requirement had dropped from 50 units a day at which she was not well controlled at all to 25 She was now easily controlled on 25 units and her peripheral neuropathy had entirely disappeared that is to say as far as symptoms were concerned She still had absent deep reflexes There was really a remarkable degree of improvement

We have been unable to find any consistent effort to treat this particular variety of insulin refractory diabetes in which a pituitary factor is at least strongly suggested with intensive radiation to the

pituitary I wonder whether anyone had had any experience with that

Shorr Did you study 11 oxysteroids?

Watson No we didn t

Sherlock Dr C L Cope at the Postgraduate Medical School in London made oxysteroid estimations on the urines of two of our obese diabetic subjects There was no increased excretion of oxysteroids in obese hepatic-insensitive diabetic individuals The results were quite normal

Gyorgy Dr Best I have been raised under the impression that in uncontrolled diabetes you have no or only a very small amount of glycogen in the liver Why is it now that we hear there is a lot of glycogen? Is there any explanation for the faulty teaching of twenty odd years ago?

Popper May I answer this question with personal experiences? I told them before to Dr Fremont Smith who encouraged me to mention them here I was also raised under the impression which Dr Gyorgy mentions About 25 years ago I transferred from Fuerth s biochemical laboratory to the Pathologic Institute in Vienna There I heard a lecture of my teacher Maresch on glycogen degeneration and he cited as a typical example the diabetic liver I told him that I had done some glycogen determinations on livers of pancreatec tomized dogs and they were practically free of it I thought there fore that he must be wrong because everybody agrees that absence of glycogen from the liver is characteristic for diabetes He con ceded that he was not well acquainted with metabolic problems but he knew from extensive personal experience that the human diabetic liver is as a rule rich in glycogen and different ideas of the biochemists could not very well change his observations This was around 1926 As a student in the Pathologic Institute I started to examine diabetic livers for glycogen Most places in Vienna at that time treated diabetics already intensively with insulin but one of the places did not do it yet at least not intensively Therefore in this transition period we examined also few untreated cases

Independent of the treatment the livers of persons dying in diabetic coma contained as a rule far more total carbohydrates as well as far more glycogen determined chemically and histo logically than the average autopsy material The total carbohydrate and glycogen concentration was higher on the average inpatients

dying in diabetic coma than in those who died from septic complications or in whom the diabetes was an incidental finding, especially in the older age groups(55) The glycogen content of the livers of persons dying in diabetic coma was equal or averaged a little less than that of perfectly healthy people who died as a result of accidents and whose livers were obtained from the Legal Medical Department But it was, as a rule, considerably higher than the common autopsy material derived from persons who had died from all kinds of diseases after an agonal period similar to that of patients dying with diabetes The point I would like to stress is not that in diabetic coma the glycogen content is higher than normal, but that considerable amounts of glycogen are present These observations have subsequently been confirmed It is rather difficult to confirm them now from autopsy material because untreated or insufficiently treated cases of diabetes are practically unavailable I know from Dr Sherlock and others that glycogen not only in the nuclei but in the cytoplasm can be found in biopsy specimens of human diabetic livers I should like to have her comments

Sherlock I think it is true in human diabetes that the glycogen content of the liver is normal or supernormal That it is also true — Dr Stetten will bear me out — that in the alloxan diabetic rat or rabbit there is a supernormal amount of glycogen in the liver (56),(57)

Stetten That has not been our experience

Best No, I don't think so

Stetten For one thing I think there is probably considerable species difference The alloxanized rats that we have seen have very little or no demonstrable glycogen in their livers On the other hand, the diabetic rabbit is reported to have normal or more than normal quantities

Best The diabetic dog also has very little

Sherlock The alloxanized?

Best The depancreatized

Sherlock That is different

Best But in alloxan diabetes and the spontaneous diabetes may have some insulin and the depancreatized dog does not any

Sherlock The depancreatized dog is likely deficient in c things

Best If you give him insulin, he gets glycogen, though

Shorr I wonder what percentage of diabetes, Dr Best, matter of altered equilibrium between insulin and principles inh ing carbohydrate oxidation and storage?

Best Dr Wrenshall is presenting his data this year at American Diabetes Association He found — I have forgotten exact number of the cases — that all of the diabetic children died had no insulin in their pancreas, all the patients who die coma had no insulin, but all the rest had lots of insulin, up to 5 60 per cent of the normal So you really could not compare th The two groups are quite different One is, from the point of v of insulin, partially diabetic, and the other is completely diab

Madden May I ask Dr Popper whether or not his experim included children who had died of diabetes?

Popper We examined only one child with diabetic coma Hi logically, much glycogen was found in the cytoplasm of the l cells and the chemically determined glycogen content was h (3 62 Gm /100 Gm), and the total carbohydrates concentra was 5 93 Gm /100 Gm wet liver In all, we analyzed over a hund cases(58), however, I should like to stress that glycogen was found in all livers There was a certain percentage, I should about 20 per cent of cases of diabetic coma, in which the li was free or almost free of glycogen If my memory is correct, first examinations on diabetic livers were done many years by Frerichs who described large amounts of glycogen which stai histologically with the iodine method Therefore, I believe t long before the present control of diabetes was known it was fi established that the human diabetic liver may contain considera amounts of glycogen

Sherlock Am I not right in saying that Dr French's work v on liver biopsy material and that the biopsies were done by Ehrlic

Popper I believe you are right that French and Ehrlich did t first liver biopsies

Best Dr Lyman Duffy and his colleagues found that the vacuol seen in the islets of Langerhans are full of glycogen

This discussion is very interesting but I fear we must no adjourn

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SECTION V

LIVER DISEASE IN JAMAICAN CHILDREN

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University College of the West Indies

I AM AFRAID THAT after previous astute investigations this paper comes somewhat as an anticlimax

I should like to give you the background of our work In my department I have myself my part time associate Dr Rhodes and a very good technician and we have begged and borrowed stains from the American Army camp and other institutions nearby and from some local laboratories we have borrowed a rocking microtome and we have access to our crowded government hospital where we have six beds

Now we have with the cooperation of the University of Pennsylvania and the Jamaican Government in 18 months studied over 120 cases We have done liver biopsies on 60 some of them serially (so we have examined 100 liver biopsies) and we have post mortem examinations on 10 †

Brandy and McFarlane(1 2) studied the problem of liver disease in the West Indies Waterlow(3) in 1948 studied 15 cases the majority of which were Jamaicans and he described a condition of edema and muscular wasting and enlargement of the liver which was showing fatty metamorphosis and he gave that the name "Fatty Liver disease in infants in the British West Indies" The same condition was described by Royes(4) the same year These three separate groups of authors more or less found a pattern of disease that is now well known Incidentally Cecily Williams(5) on the Gold Coast described a condition which she called "kwashiorkor" with which this society is very familiar as Dr Davies(6) was here last year And a similar condition was described

* Acknowledgment This work was supported by grants from the Lederle Laboratories and the Josiah Macy Jr Foundation

† The Microphotographs came from 20 of the 60 cases biopsied

Clinical		Age	Diet	Biochem	Histol	% of Total 108 Cases
I	Starved Liver Enlarged and Firm	5/12 - 15/12	Low Prot Low Cals	Generally Abnormal	Collagenosis With Fatty Metamorphosis	5%
II	Look Well Liver Enlarged and Hard	4/12 - 16	Low Prot Adequate Cals		Collagenosis	65%
III	Poorly Nourished Liver Nodular or Contracted	2 - 17	Low Prot Low or Adequate Cals		Fibrosis	30%

Figure 1

by Dr Trowell which he described as "malignant malnutrition"(7) or "infantile pellagra and by the Gillman brothers(8) and so on

Now I want to comment on the statement by Dr Fremont Smith that we may be bound to the concept of unicausality I must confess that I was more or less bound to the concept of fatty liver disease of the West Indies It was *more or less handed to me on a platter* and after all I had been on the Gold Coast which was the stamping ground of Cecily Williams and Professor Maegraith

Maegraith (Interposing) Not at the same time

Hill and I had seen fatty liver disease in Bagdad I had seen it in the hills in Simla I also saw it last year in Java

So we came and started this investigation and after a short time we came to the conclusion that this fatty metamorphosis was not the true picture in Jamaica Well more of that anon

Figure 1 will give you some idea of our clinical findings as shown by Dr Rhodes Dr Rhodes is a very astute clinician She can differentiate our cases into three clinical types and yet we find there is an underlying pathology all have the same pathology We have now studied over 120 cases The figures in the last column refer to actual percentages

In this chart type II represents the classical case and about 65 per cent of our cases are like this (Figure 2) First of all they look well They are apparently well nourished They are chubby They ha [illegible] old an [illegible] lder th [illegible] the inevitable umbilical hernia very seldom do they show edema and if they show edema it is due to the same causes as the edema from an abdominal tumor pressing on the legs She has an edema of the legs a dependant edema

She is presented very often acutely ill She is fretful and she is ravenously hungry

Sometimes they come — very few of them — pyrexial because they are very prone to upper respiratory tract infection but that is not the classical picture They come because they are ill and sometimes because they have a very protruding abdomen The left lobe of the liver is firm and enlarged as often as (and sometimes larger than) the right lobe Sometimes the enlargement is such that the

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Sometimes they come — very few of them — pyrexial because they are very prone to upper respiratory tract infection but that is not the classical picture They come because they are ill and some times because they have a very protruding abdomen The left lobe of the liver is firm and enlarged as often as (and sometimes larger than) the right lobe Sometimes the enlargement is such that the

upper surface is practically horizontal and can be almost right down to the pelvic brim

And when I say 'she' I should not say that because the sex distribution is the same And I should also mention that racially they are of African extraction Chinese extraction and East Indian extraction They belong to one low economic level, as I shall tell you later, as regards the diet But there is no racial discrimination in this disease

When they are first tapped, their ascitic fluid has a high protein content just below about 3 per cent of protein After they have been tapped then the ascitic fluid is watery and remains so, of low protein content



FIGURE 2 A Jamaican child with liver disease aged 2 yrs note the apparent appearance of good nutrition the enlarged liver and ascites A typical case

When we see them like this we have a history very often of maybe a week up to about six months When they are in the hospital they often run a temperature for about four or five days and we do not know why We investigate them carefully eliminating the upper respiratory tract infection to which they are very prone Their white count does not go up Their ages vary from four months to sixteen years the earliest being four months They have a low protein diet and adequate calories

Histologically we find – and I am sticking my neck out here but I was given permission to do that by Dr Fremont Smith – a condition of collagenosis Well that is the 65 per cent and that is the classical picture (Figure 1 type II)

Now only five of our cases – I put it down as 5 per cent – 5 out of 120 showed another condition These cases (Figure 1 type I) which actually showed fatty metamorphosis were starved and edematous These were edematous all over a starvation edema Their ages varied from 5 to 15 months They had various dermatoses crazy pavement skin receding of the hair and skin conditions which are described in "Kwashiorkor" by Cicely Williams(5) We never saw any evidence of deficiency in this main classical group (Figure 1 type II) In the cases that had the starvation edema the liver was large and soft and they had a low protein diet and a low calorifi ... I) showed fatty metamorphosis ... out of five cases that had under ... show you

The fifth case we did not see The specimen was just a fragment Really I should not report it

The next type of case (Figure 1 type III) was 30 per cent These were poorly nourished They had a nodular liver or a retracted liver and their ages varied from 2 to 17 years They were on a low protein diet low or adequate calories and in the histological picture the overall picture was fibrosis But in point of fact as I will show or hope to show in all these cases (Figure 1 type I ... ession from serous exudation ... e cases (Figure 1 type II) ... (Figure 1 type III) it was ... [illegible]

In this slide (Figure 3) I will give you some indications of the age incidence The peak age incidence as you can see is from about 1 to 3 to 4 years and then seems to fall off

The black parts here are Dr Rhode's clinical diagnoses (which are *practically all supported histologically*), of the main, well nourished, classical type with a firm liver This lightly shaded part here is where there was starvation and fatty metamorphosis and these stipples were those which she found poorly nourished and *she diagnosed as cirrhosis,* and by the way, *these* cirrhosis cases or fibrotic cases, if they died, were associated with such conditions as hemorrhagic esophageal varices and so on

Summarizing We had five cases of fatty metamorphosis (Figure 1 type I) under 2 years old one case was a classical kwashiorkor

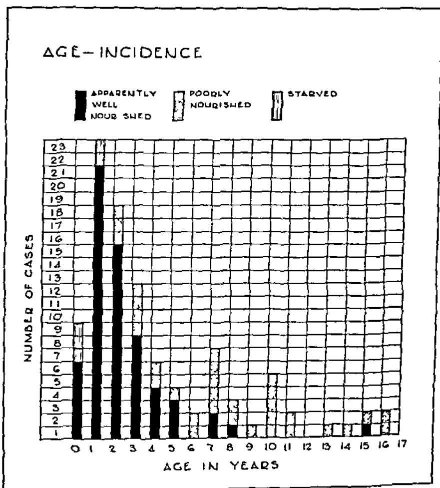


FIGURE 3

We had the majority of our cases in the 0-4 years old group (the youngest was 4 months old), and they were mostly our classical clinical type of case (Figure 1 type II), and we had a number of cases in the older age groups (up to 17 years) in which the main clinical type appeared to be fibrosis (Figure 1 type III)

Now, actually, *these are selected cases* They are selected because these cases were brought to us by the mothers because the children were ill Only occasionally did we get one of the other members of the family, although we asked, 'Is there anyone else with enlarged liver in the family?' and that is how we got these few older groups

There is in Jamaica probably much more asymptomatic "collagenosis" or fibrotic liver disease, cirrhosis if you like, in the older age groups Our cases are generally seen as much younger patients *who are acutely ill*

It seems that when you examine these cases histologically, you get an entirely different picture as to distribution In the under one year group, you find serous exudation, "collagenosis" and fibrosis In the child four months old who had been fed on the breast – with a low protein diet, there was certainly portal activity and early fibrosis In these very earliest cases you have collagenosis and fibrosis, and in these later cases, also, you can see collagenosis and fibrosis but, of course, the fibrosis predominates in these later age groups So this condition which is taking place in the liver is taking place all through these age groups The incidence, as I said, seems to be peak from 1 to 4 years of age, but I said you have got to modify that statement, for these cases are selected

In the next slide (Figure 4) you will see something of these children's diets

We have taken, for purpose of comparison, five normal Jamaican children – they were gotten from the Child Welfare Clinic and from the surgical ward – and they were compared with ten of our cases In about a dozen to fifteen of our cases, we investigated their diets fairly thoroughly We went to their homes, or at least Dr Rhodes did, spending from 6 to 14 days, and made a thorough investigation of what the patient had eaten the day before

If anything, because of the natural pride of the Jamaican, our figures as regards the diet are higher than they actually were I n

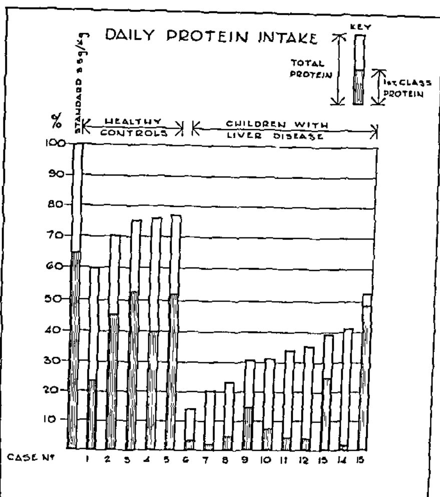


Figure 4

only tell you that from the economic standpoint a woman with four children will live on one dollar a week, so you can just imagine the amount of food that is bought by these people

As regards the protein intake, we have taken standards from the National Research Council, and that is 3 5 g of protein per kg of body weight a day

We always take a very careful history, and we have investigated as I say, twelve to fifteen, or so, and the whole pattern is the same that is, there is much less protein taken in the diet of our liver cases than in our control, so called healthy Jamaicans

If you take No 2 and compare it with Nos 11 and 13 those 3 are all three years old, yet this one (No 2) takes about 35 g of protein a day, this one (No 11) takes 10, and the other (No 13) takes 17 or 19 9 g of protein a day

So the first point is that they have a deficient protein diet

The next thing is that you must take into consideration the variety of the diet These 5 controls and other controls which we generally have investigated, take on the average (other than breast-fed children) somewhat in the order of 20 articles of diet in a week

Our liver cases generally take less than 10 articles in their diets Their diets are monotonous There is no variety whatsoever in their diet

In this chart (Figure 4) in the shaded parts are represented the first class or animal protein

The source of their animal protein is interesting Out of these 10 liver cases, almost the only source of animal protein is condensed milk *In the control group is a source of animal protein* they have beef and sometimes eggs and so on

Now, when you come to the actual source of vegetable protein
ve all sorts
vegetable
and green
bananas, with little variation from this

I should like to read out two diets

WINTON ROACH AGE 11 MONTHS

No of feedings 5 per day, 4 breast feedings per night The feedings during the day are as follows First feed mint tea with two teaspoonsful of brown sugar and four teaspoonsful of condensed milk Second, strained Quaker oats with condensed milk Third strained Quaker oats with condensed milk Fourth strained Quaker oats with condensed milk Fifth condensed milk diluted with sugar added, one teaspoonful of condensed milk per feeding

Breast deficient in protein fat and carbohydrates

DOLORE CHIN AGE 1 YEAR 3 MONTHS

No of feedings 5 per day 1 at night

First meal mint tea with 1 teaspoon of sugar no milk Second, corn meal Third corn meal Fourth corn meal Fifth mint tea with 1 teaspoon of sugar

During the night mint tea with 1 teaspoon of sugar

Daily milk consumption half a pint of cow's milk was mixed up with the corn meal

Now, the other interesting thing about these very deficient diets is that the normal Jamaican – and now we go to figures which were compiled before the war, and they still stand, and Professor Mackay in the Department of Physiology will bear this out – in spite of the fact that he has a deficient diet compared with our North American children and our English children, he has the same height The average height of the Jamaican is the same, age for age, as it is in North America, yet when we come to our liver cases, they are all stunted

Now, so much for the clinical side

As regards investigations, we have done batteries of liver function tests on all our cases I should like to slur over this, quite candidly, *because our biochemical department is not yet started* We are functioning with a photo colorimeter in which we have voltage [illegible] to 130 volts, so our results, [illegible] wever, the pattern that we [illegible] f our cases, the albumin is less than 3 5 g per cent There is a deficiency in the albumin content of the blood The other thing is that in 40 per cent of our cases – and we have just started doing this, have lowered serum choline esterase The flocculation tests are equivocal The one-minute bilirubin and total bilirubin are not very clear In other words, in the liver function tests, the patterns are not clear at all *Sometimes even when the child is acutely ill, it seems to give* normal liver function tests We do flocculation tests, lipid determination, and so on, and we cannot get anything out of them

I am sorry I really cannot discuss this in detail except that we seem to have a lowered serum albumin, and we also see a lowered serum choline esterase

Now, as far as other investigations, only three of our 120 cases show a positive Kahn All of our cases are routinely tested for malaria, and only 4 on serial examinations have been positive for malaria And rather interestingly, a case which I have excluded from our series – it has an enlarged liver, but did not give a positive blood malaria – exhibited a beautiful extra erythrocytic schizont in one of the liver cells Actually a classical picture We have six sickle cell anemias Ankylostomiasis which is common in the Island, is generally not present in the examination of the stools we have done here, and only about four of the cases have given positive stools for this parasite I say this because in Curaçao they have

fatty liver disease but in Curaçao they do not have liver cirrhosis and they do not have malaria nor ankylostomiasis So we have been looking out for those parasites in particular

We have one case of tuberculosis pulmonary tuberculosis among these We have not done any skin tests as regards viral hepatitis and the investigations that we have done as regards other etiological agents have been very incomplete

Now as regards treatment we are treating these patients in the Government Hospital wards As soon as the child comes in he is put on a high protein diet and we cannot stop that Therefore we have not any really good controls The high protein diet is given 20 to 30 ounces (an Imperial pint to a pint and half) of milk two eggs possibly some little meat and plenty of vegetables That is a high protein diet for the Jamaican child

Our classical cases when they come in are voracious in their appetites I mentioned this yesterday They want protein and they simply lap it up They are not concerned with cereals at all They just push them away They do not eat them They go for the protein They do fairly well after two to four months I want you to remember that figure Their livers recede The ascitic fluid dries up and because of the pressure for the hospital beds they have to be discharged Of course it is hard to hold the child for two to four months but they do react very well to high protein diet and that of course is in line with work all over the world – India Africa Bagdad and Java

In our fatty cases the five fatty cases we had showing generalized nutritional edema with a deficiency dermatosis and so on you could not get them to eat They had gone to the point where food was of no interest In one case where we did give high protein and forced it we practically killed the child But the others we had to nurse with half strength milk until they got to the point where they could take decent meals and then they did very well the fat disappeared from the liver and the liver retracted and so on

We have tried various other things Some of our treatment is out patient treatment and it is not good treatment because you give stuff to these women they will take it away but they will either put it in the cupboard or they will sell it to their next door neighbor Anyway the child does not get it Such has been our experience with vitamin B Therefore I cannot report for or against

our cases on vitamin B_{12} Such also has been our experience with aureomycin I cannot really give you our results

We have had a series of six cases on animal protein factor For three weeks the six cases did extraordinarily well They all put on weight, and, by the way, these cases are all underweight Their liver retracted After three weeks they stopped putting on weight They hit a kind of plateau Then some of them started losing weight and then some of them actually started getting enlarged livers We did not investigate them We did not do a liver biopsy to see if those livers had become fatty It is a pity, perhaps, that we did not investigate them But again it was not a very good experiment

Dr Rhodes then started treating the cases with Ventriculin, and we have gotten the most extraordinary results If I hadn't seen them, I would not have believed it It did not matter whether they had been on high protein diet for two months before or whether they had just come into the hospital The facts are that within 4 to 21 days, those cases got clinically well Those were actually six of what we would call clinical cures

The formation of ascitic fluid was reduced or did not reform The liver receded They were able to be discharged from the hospital in three weeks Seven of them did remarkably well, four had gross fibrosis, and there is one boy who has been in the hospital for years and years with an enlarged liver and with a tremendously distended belly which had to be constantly tapped We put him on Ventriculin, and in a matter of two weeks the ascitic fluid dried up, the liver retracted Why that happens with Ventriculin, I do not know

Hanger Ventriculin is superior to crude liver?

Hill It is

Gyorgy It is hog stomach

Hanger I say is it superior to crude liver? Has it a specific action?

Gyorgy Apparently

Hill Well, incidentally, as an aside, I remember Ventriculin from the Addisonian type of anemia As I remember, Ventriculin was a gray powder, and Dr Aub, who has come on our Research Staff part time, from Germany, tells me that in Germany the Ventriculin he knew was a fine chocolate powder The Ventriculin we are using

from Parke-Davis now is a coarse granular, light brown powder I can only describe it as blunderbuss therapy I do not know what is in Ventriculin – I mean what the amino acids are I know its action is subject to speculation

I remember the Gillman brothers(9) said they had used Ventriculin with some success, and then they tended to withdraw that statement later on I wondered whether the sort of Ventriculin they had was just dry hogs' stomach

Best Are these active enzymes in Ventriculin?

Neefe How much did you give?

Hill The dosage varies from 15 g to 30 g per day for 14 days

Watson Might I ask Dr Hanger what he meant by a more specific action of Ventriculin? What were you referring to?

Hanger As I understand it, the two were quite replacable in the treatment of pernicious anemia and I was just wondering whether Ventriculin had some special action which crude liver did not have in the way of ingredients or enzymes or some specific amino acids, just the things that had been mentioned

Watson Oh, you were asking I understood you to say that it did have

Hanger No, I was asking whether it had more specific beneficial effect than crude liver

Hill I do not know Wilkinson(10) in Liverpool, claims that it is better for pernicious anemia with subacute combined degeneration of the cord That is because of its hematopoietic action

I do not know whether I should mention here that blood examinations were done in all these cases, and they all showed some mild degree of hypochromia

Addison's type of anemia in the West Indies, in general, is very rare

Shorr No iron in the liver?

Hill No

Watson Did I understand that you had tried B_{12} in a series?

Hill Yes, not a scientifically controlled experiment It is practically impossible for us to do it really

Watson I wondered why it is more difficult to give that than Ventriculin

Hill Well Ventriculin works The little bit we had of B_{12} as Dr Gyorgy will tell you did not seem to work

Watson One wonders whether the action of Ventriculin was due to B_{12} That is an important question it seems to me

Gyorgy I have to admit as Dr Hill said the B_{12} studies were outpatient observations and they were not well controlled You will admit that

Hill Yes

Gyorgy Therefore I do not want and cannot say too much However a few patients got B_{12} on the ward and according to Dr Hill and Dr Rhodes they did not respond as dramatically as on Ventriculin

Hill Really our therapeutic trials are not scientific I only give them because of what Dr Fremont Smith said yesterday But I will say that I am most struck with Ventriculin and its effect—a most extraordinary effect

Neefe Is amebiasis prevalent?

Hill No amebiasis is not prevalent in Jamaica

Maegraith Were there any changes in hair color in these children?

Hill Well first of all there are quite a number of red headed people in Jamaica of African extraction But the five cases that showed fatty metamorphosis did have receding hair lines some times rather grayish whitish hair There was one classical Kwashiorkor the red dermatoses straight hair and rather pale hair but Mackay in the Physiology Department who has done a nutritional survey on many normal people tells us that they turn out red haired boys quite a lot

Dauphinee Was there any response as far as the red cells and hemoglobin are concerned to the administration of Ventriculin?

Hill I really cannot answer that one I have it in the records

Dauphinee Was there any reticulocyte response to its administration?

Hill I am afraid I did not do that

Smetana Dr Hill, your study reminds me of Dr Davies' cases observed in Uganda Did you see his material?

Hill I am familiar with Dr Davies (6) work and all the Uganda work, including Trowell s(7), and I just happened to glance at your transactions which mentioned that

Well, again I am asking for trouble because Professor Maegraith is here I was in Accra about two years, and we knew of Cecily Williams' work(5), Williams worked up in Kumasi (I believe) where the economic level was not very good, and she saw these cases of kwashiorkor Around about Accra, I never saw a classical case of kwashiorkor They appeared to have good nutrition and hard, firm livers Those that I saw, incidentally last year in Bagdad – I'm sorry I did not bring a photograph of those with me – they were just the same as in Jamaica, but I was told they had fatty livers on biopsy and they responded to blood transfusions and to milk The same is true in Simla, the same is true in Java

Maegraith In Assam I noticed that the changes in the color of the hair were very common That seemed to be a very characteristic part of the syndrome I picked up what I regard as one of the best specimens I have ever collected in a hospital right up on the borders of Assam There was a small child who had been in and out of the hospital about four times with this condition Each time he had come in his hair was white, or gray When they got him into the hospital and fed him up on protein, his natural hair color came back When he went out again and the syndrome returned, the hair went white again Then when he came back to the hospital, it went black again All this time his mother never cut his hair Eventually, when I got him, his hair was about a foot long and it had four different white places and the rest was black It was the most beautiful picture I have ever seen I have it mounted in plastic

Neefe Does the treatment of the fatty liver group with Ventriculin not produce the ill effects that you encounter when you try to feed that group?

Hill We have not treated any of the fatty livers with Ventriculin There are only five, and they are so rare We have not come across them this year, just last year

Neefe Then it is your "classical group" which responded to Ventriculin?

Hill That is right

Neefe Then you do not know what the effect would be in the group in which overfeeding seemed to make them worse initially?

Hill As I will show, the fatty metamorphosis, to mix my metaphors, is just a red herring In these fatty cases I will show you

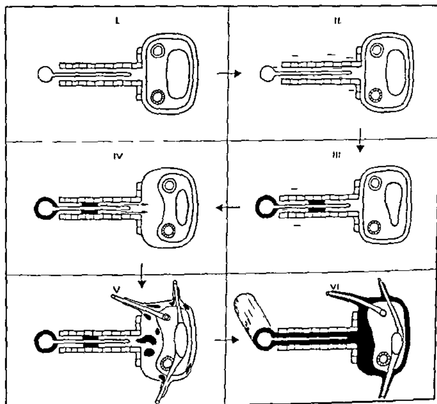


FIGURE 5 Diagram showing the various stages of the pathogenesis of hepatic fibrosis
(i) ... the hep t c laminae within ... own ... e of Disse (lacuna)
(ii) serous exudation in perisinusoidal space of Disse
(iii) Deposition of coagulum (a) around centrilobular vein and also (b) within the space of Disse
(iv) Distension of space of Mall following further serous exudation subsequent to the deposition of coagulum
(v) Deposition of coagulum from the serous exudates present in the space of Mall and in the sheaths of the intra and interlobular vessels
(vi) Late stage of deposition of coagulum which becomes fibrosed by the invasion of fibroblasts

fatty livers that also show "collagenosis and all the three gro are clinical manifestations of the same underlying pathology

Figure 5 is a diagrammatic illustration of what happens

Incidentally, I am quite influenced by Elias (11) work, and a by Popper's(12) work As you will see that will tend to come in my talk

Figure 5 I represents part of a liver lobule It consists o centrilobular vein and a sinusoid The sinusoid lies in a lacu which is lined by the liver cells making up the hepatic lamina you may call them under the old terminology hepatic colum Between the sinusoid and hepatic laminae is the perisinusoi space of Disse The portal triad consists of bile duct artery a portal vein and at the edge of the triad is the potential space Mall the space of Mall connects with the perisinusoidal space Disse

We think that the first – and I will try to show this – the f manifestation of this disease is a serous exudation around the cent lobular vein and in the spaces of Disse (Figure 5 II) That actu

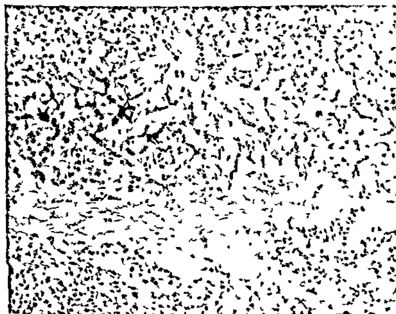


FIGURE 6 Serous exudation in liver of child aged 1½ yrs H & E x 150

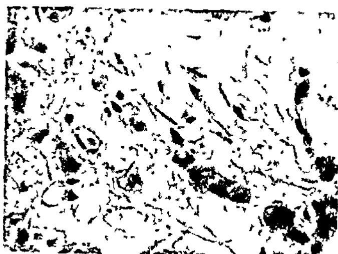


FIGURE 7 Serous exudation in the spaces of Disse Same case as Figure 6 H & E × 400

FIGURE 8 Liver of child aged 2 yrs showing fibrillary collagen from previous attack and serous exudate from current attack Modified Mallory connective tissue stain × 600

happens in about 25 per cent of our cases At a later stage or possibly not a later stage possibly primary there is a deposition (by H & E stain) of an eosinophilic coagulum around the central vein or within the space of Disse (Figure 5 III) This happens in 80 per cent of our cases and we have studied 60 cases

Figure 5 shows a typical case As I said 25 per cent will show this There is a serous exudation with flattened or atrophied cell columns Here and there can be seen centrilobular veins or sinusoids — it is hard to say which is which and the spaces of Disse show much distension with serous exudation In some of these cases I have done 80 serial sections

Sherlock Is that a biopsy?

Hill All this material that you are seeing today is obtained by liver biopsy

In Figure 7 you will see Figure 6 at high power

Figure 7 shows flattened or atrophied liver cell columns and 2 sinusoids with swollen endothelial walls the perisinusoidal spaces of Disse are distended with a serous exudation



Figure 9 Focal areas of serous exudation and coagulum in liver of child aged 11 months Modified Mallory connective tissue stain × 60



FIGURE 10 Serous exudate and eosinophilic coagulum in liver of child aged 2½ yrs Note the swollen endothelial cells of sinusoid in centre and that several areas of coagulum are fibrillar H & E x 600

In Figure 8 you will see a serous exudation superimposed upon the deposition, by a previous attack, of an eosinophilic coagulum This slide is a Lendrum modification of Mallory's stain We think that this is collagen ' It gives us all the tinctorial reactions of collagen It is a protein precipitate when we fix it with our fixative, and it has already taken on a fibrillar character The main point about this slide is that around the vessel there is a collagenous cuffing

Smetana Is this material birefringent?

Hill No! It is, as I shall tell you later, not birefringent

Figure 9 is a low power showing that the lesions are focal This is stained by Mallory's stain or Lendrum's modification and there is collagen around the central lobular vein The normal liver not involved in the serous exudation shows some slight compression and mark you the first two slides I showed were in a child 18 months old with the liver about three fingers breadth below the coastal margin — a large, firm liver This child was 11 months old and the liver was considerably enlarged

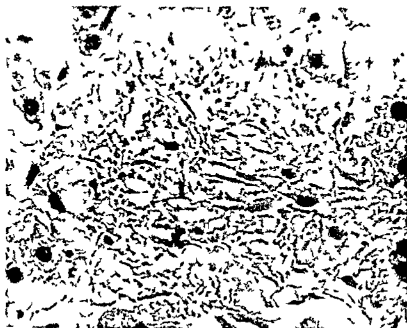


FIGURE 11 Serous exudate and eosinophilic coagulum in liver of child aged 2 yrs H & E x 600

Figure 10 will show — this child was 2½ years of age — a serous exudation within the liver and a swollen sinusoidal endothelium but there is now deposition of an eosinophilic coagulum which gives all tinctorial reactions of collagen and in Figure 11 you will see in another case age 2½ years the serous exudation and deposition of an eosinophilic coagulum

In Figure 12 you will see a serous exudation This child is 1 year and 3 months There are flattened liver cells but here is a deposition of what we think is collagen which has gone fibrillar The point is that if you see the H & E section you can see the laminations just as in a tree This is the third attack as it were

Hanger And that was the third attack?

Hill It had two subsequent attacks on top of that There is no collagenosis immediately adjacent to the centrilobular vein

In Figure 13 you will see a central lobular vein showing collagenous cuffing There is some fat there There is fat in 5 per cent of our cases



FIGURE 12 Li er of ch ld aged 1 yr 3 mos showing (a) collagen cuffing of centrilobular ve n from previous attack and (b) serous exud t on from current attack Modified Mallory connective t ssue stain x 150

I will go on to the staining reactions in a little while

Figure 14 will sho
lobular vein there is
on this case This l
vessel with a collagenous cuffing arising from the intercalated vein This is an H & E stain Figure 15 shows the same block cut about 100 μ deeper it shows collagen cuffing extending through the liver around the two tributaries of the vessel demonstrated in the previous figure

When we first saw these cases we were puzzled We got focal scarring in the lobule We could not understand that until we started doing serial sections and every time we could trace the scarring to a vessel of some sort whether it was the main centrilobular vein or whether it was the sinusoid

In Figure 16 you will see this eosinophilic coagulum clearly demarcated in the spaces of Disse This may be as an aftermath of a serous exudation It is very hard to be actually certain The serous

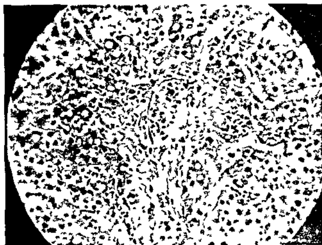


FIGURE 13 Eosinophilic coagulum around the centrilobular vein in a liver of child aged 1 year Note there is some fatty metamorphosis H & E × 400

FIGURE 14 An intercalated vein with a tributary showing a collagen cuff in a liver of child aged 11 months H & E × 150

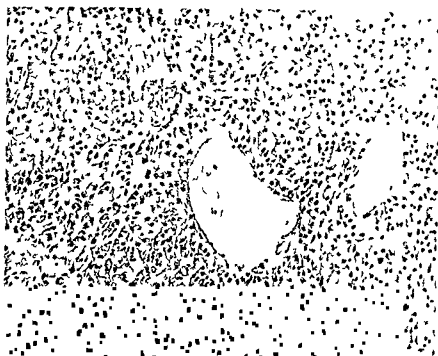


FIGURE 15 Serial Section of Figure 14 but cut 120 μ deeper two tributaries of the tributary vessel in Figure 14 both show collagen cuffing H & E x 150

exudation is presumably a proteinous fluid — and later you may have a precipitate out of it — it may go in the form of a gel I do not know Whatever the cause whether it is an aftereffect or whether it is a primary effect it happened in 80 per cent of the cases

The Figure shows a sinusoid and also liver cells there is not too much wrong with those liver cells (I will talk about liver cell damage afterwards), and between the two is this eosinophilic coagulum

Figure 17 is from a boy aged 4 There is some eosinophilic coagulum and we can discuss that in a moment when I show the high power There seems to be no serous exudation There is however here if I may borrow the popular song wide open spaces" and this is a common finding there are many blown up sinusoids I see two or three and I will demonstrate those later on

Whether there is a blocking due to this eosinophilic coagulum I do not know We do not know why we get these wide open sinusoids

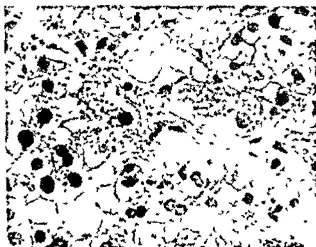


Figure 16 Eosinophilic coagulum in the space of Disse in the liver of a child aged 7 yrs H & E x 400

Figure 17 Deposition of eosinophilic coagulum in serial places in liver of child aged 4 yrs H & E x 150

In Figure 18 you will see a beautiful illustration of this eosinophilic coagulum Here is a sinusoid with swollen endothelium, here are liver cells which are flattened Into the space between sinusoid and liver cells is poured a waxlike substance When I took one look I said, '*amyloid*,' *but subsequent staining showed it was not It is not metachromatic, negative to Congo red, iodine green,* not double refractile It is not para amyloid It stains reddish with Mallory phosphotungstic, blue with Mallory's connective tissue stain, eosinophilic with H & E It is not fibrin tinctorially, it is not amyloid, it is not para amyloid and it is not dissolved by trypsin That is about as far as we can go at this moment, but it is some thing within the spaces of Disse

In Figure 19 you will see the next section to Figure 18 stained by Mallory's *stain The coagulum gives a blue tinctorial effect* There are no cells within it and there are no fibroblasts

In Figure 20 there is a reticulin stain of the next section to Figure 19 The first thing is that the blobs of coagulum stain gold and yellow, and the reticulin fibers stain black It is a modified Foot Wilder stain I should have also said that the coagulum never gives a reaction for hemoglobin, and there is no suggestion that there is any blood content here What we can see here are swollen reticulin fibers to which are adherent blobs of coagulum which we think is collagen or pre collagen

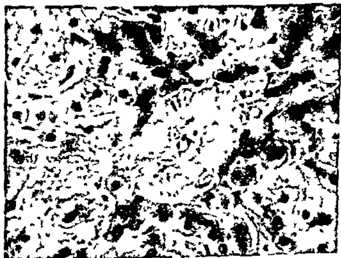


FIGURE 18 Shows waxy like eosinophilic coagulum in the perisinusoidal space Section is taken from same block as Figure 17 The coagulum gives all the tinctorial reactions of collagen H & E x 400

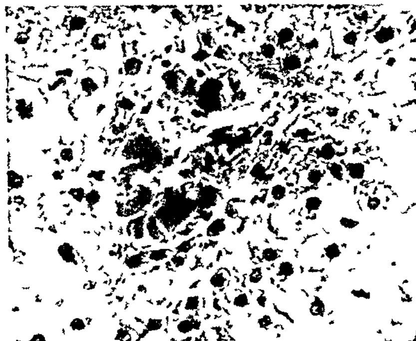


Figure 19 Serial section of Figure 18, 16 μ deeper and stained with Mallory's connective tissue stain. Note the blue staining coagulum in the perisinusoidal space; the sinuso[…] is clearly seen. x 600

We know from the work done I think at the Rockefeller Institute by Porter and Vanamee(13) that you can get fibrillar collagen from protofibrils without *intermediate* fibroblasts. There *are no* fibroblasts here. One thing *we* are weak on in our *investigation* at this stage is whether this is a *deposition* of a *protein* on to reticulin fibers already in the perisinusoidal spaces or whether there is a proliferation of the *reticulin* fibers. That we don't know. We have just started this study.

Though we have been working against many difficulties, I have on *my* staff a first class technician by the *name* of Baker, who has done Mallory's phosphotungstic *stain on this* — and it is not usually done on the liver, and the coagulum with this stain as large *reddish* blobs, but to our amazement we found, using Mallory's *phospho*tungstic stain (under high power), that although the connective *tissue* fibers of the perisinusoidal spaces stained the brown they had as it were adherent *to them* little



FIGURE 22 Shows high power view of Figure 21 The eosinophilic coagulum has been deposited in the spaces of Disse throughout the lobule — "perisinusoidal collagenosis H & E x 600

In Figure 24 you will see these wide open spaces but there is collagen deposited very patchily — not within the portal tract — this is within the lobule

In Figure 25 you will see a high power of Figure 24 Within the perisinusoidal spaces are collagen fibers and there are some fibroblasts but there is a wide open space probably a sinusoid But notice the collagenous fibers in the perisinusoidal spaces This appearance is actually at much later stage

Figure 26 is a case which has been going for some time you see collagen cuffing of the centrilobular vein collagenosis spreading out into the lobule right from the center this is a very very common finding and I will show this in the reticulin stain in Figure 27 The reticulin shows this cuffing wh spreading out And if I did a Mallory stai could shov osis collagen cuffing around the cent ; perisim similar to that shown in this reticulin



FIGURE 23 Serial section of Figure 21 8 μ deeper and stained with modified Foot-Wilder reticulin stain Note the marked perisinusoidal reticulinosis x 150

But you will say to me That is not the end picture you see in Jamaica , because 30 per cent of our cases clinically show marked hepatic fibrosis and when we have hepatic fibrosis we have activity of the portal triads, and you will remember from Figure 26 that there was some activity in the portal triads

Here again we are making assumptions but I will attempt to show what is happening referring to Figure 5, the normal liver unit is shown in I serous exudation in II and deposition of coagulum in III This coagulum is blocking the spaces of Disse (If you have ever tried to blow out an egg through a very small hole in the shell, you will understand that a gel takes some moving once it is there) We think it has been deposited in a series of attacks, because we are sure that these cases in subsequent attacks drain fluid up into the spaces of Mall (Figure 5 IV) Once that fluid that serous exudate goes into the spaces of Mall you get a deposition of eosinophilic coagulum in the spaces of Mall (Figure 5 V) And now I have complicated our picture because I believe that you have interlobular and intralobular vessels as described by



FIGURE 24 Shows perisinusoidal collagenosis and distended and empty sinusoid in a liver of a child aged 1 yr 5 months Modified Mallory connective tissue stain x 150

Ellis(11) All these vessels must go through the spaces of Mall and to some extent they must carry with them a sheath whatever that is and I believe this serous exudate goes up into this sheath It goes into the spaces of Disse and down in the direction of the portal triads into the space of Mall and from there up the sheaths of the intralobular and the interlobular vessels Subsequently the eosinophilic coagulum becomes deposited (Figure 5 V) and so you have serous exudation followed by collagenosis Finally, the col … start that fibrosis … r and generalized

Popper May I interrupt here with a question?

Hill Surely

Popper I did not quite understand the blockade you are referring o Do you mean that fluid is prevented from flowing toward the portal canal or away from the portal canal?

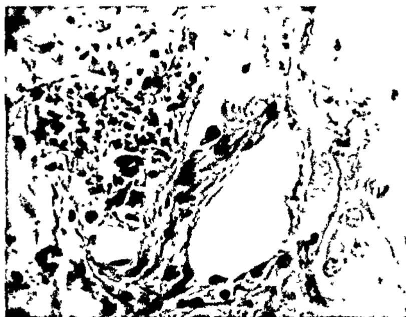


FIGURE 25 High power view of Figure 24 showing empty distended sinusoid and perisinusoidal collagenosis

Hill Well, taking Elias' picture or anybody else's, the hepatic laminae eventually must reach up to the portal triad, and the spaces of Disse, presumably connect with the spaces of Mall There is a serous exudation going on into the perisinusoidal spaces, this serous exudation spreads out in all planes for the different lacunae interconnect to make up the hepatic labyrinth I have shown in Figure 9 that the areas of serous exudation are circumscribed This confinement of the exudation to focal areas may be due to the deposition of eosinophilic coagulum restricting the further spread of the serous fluid If this happened and taking Figure 5 IV as an example, the eosinophilic coagulum in the space of Disse will prevent any new serous exudate from flowing in the direction of the centrilobular vein and rather tend to force the flow into the space of Mall in the portal triad so that the space of Mall is distended Further I believe that this is the natural flow of tissue fluid, i e from lobule to portal triad I have noticed that the perivascular lymphatics within the portal triad are very often distended and this would tend to support that drainage is in the direction I have indicated



FIGURE 26 Shows collagenous cuffing of centrilobular vein in the liver of a child aged 4 yrs Note there is also portal tract activity which has led to perilobular fibrosis H & E x 150

Popper In which direction do you assume the tissue fluid in the Dissé spaces to flow?

Hill Well, I do not know As a histologist, I see in the spaces of Dissé, serous exudation My presumption is that it is coming from the vessel

Popper But in what direction is it flowing into the Dissé spaces?

Hill From sinusoid to perisinusoidal space, and then subsequently it goes in the direction of the space of Mall It is also going in that direction, don't forget, because there are connections between different lacunae which are possibly blocked by coagulum

Popper Where, specifically, is the blockade located?

Hill The blockade is in the space of Dissé and makes the peripheral part of the space of Disse and the space of Mall a closed space, it no longer connects with the area around the centrilobular vein in the center of the lobule or with the rest of the hepatic

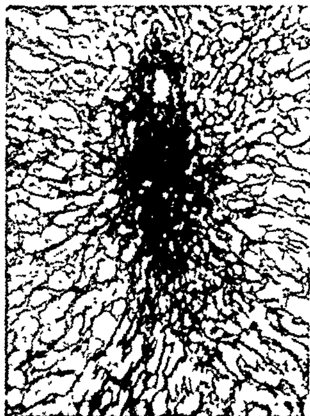


FIGURE 27 Serial section of Figure 28 8 μ deeper and stained with modified Foot Wilder reticulin stain Note the cuffing around the centrilobular vein and the extension from this into the lobule of the reticulin fibers along the perisinusoidal spaces x 600

labyrinth In any case this area may be further blocked by the deposition of a coagulum around the centrilobular vein

Diagrammatically Elias(11) has connections between the different lacunae to make up his "labyrinth hepatis" and my diagrams are two dimensional whereas the actual liver is three dimensional of course, my hepatic columns in the diagram represent sheets of liver cells agreed?

Popper Yes

Hill If fluid is poured out into the perisinusoidal spaces it will pass in all directions in the hepatic laybrinth If this labyrinth of

perisinusoidal spaces is blocked in many directions by the coagulum then the flow of subsequent serous exudation must be in the direction of the space of Mall causing its distension

Incidentally I am using the term coagulum very loosely it is only a coagulum histologically because we precipitated it with our fixative fluids It may be in fact a gel I gave the example of blowing the white of an egg through a hole that is very small

In Figure 28 I will show a space of Mall containing a serous exudation Now this happens in 50 per cent of our cases It may be said as I said when I looked at the first one or two Artefact but in this actual case in point of fact it happened in several not all but in several of the spaces of Mall

The other thing is that this serous exudation in the space of Mall is never in an area where there is a lot of serous exudation within the lobule and that is understandable because if there were lots of serous exudation in the lobule generally it probably would squash the space of Mall



FIGURE 28 Serous exudate distending a space of Mall of a portal triad in a liver of a child aged 2½ yrs H & E x 400

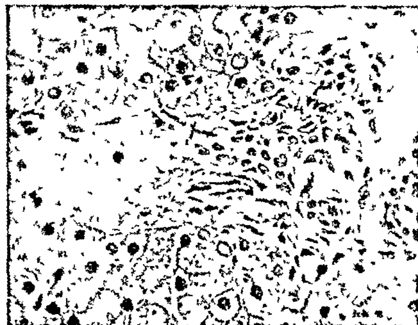


FIGURE 29 Serous exudate distending a space of Mall in a liver of a child aged 2 yrs H & E x 600

Figure 29 is that of another child it shows the portal triad with the space of Mall containing a serous exudation

Figure 30 also shows a portal triad with serous exudation And this is where the black and white photography falls down There is an eosinophilic coagulum In the H & E it is quite dramatic as if there were a precipitation of exudation going on

Figure 31 shows one of our fatty cases with "collagenosis" This is a Mallory connective tissue stain and there is a portal triad with a blown up space of Mall

Hartroft Was the fatty change most marked in the centrolobular region?

Hill I would say it was mainly central here

This case responded very well to high protein diet and subsequent biopsies of which there have been two show "collagenosis"

Figure 32 shows a portal triad with serous exudation and going up into the lobule extending from the space of Mall is eosinophilic coagulum

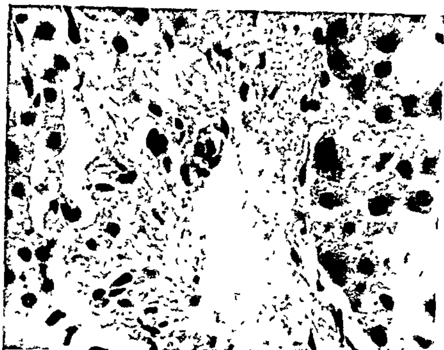


FIGURE 30 Serous exudate distending a space of Mall which also shows the early deposition of eosinophilic coagulum From liver of child aged 11 months H & E × 600

In Figure 33 you will see — and this child was aged 4 months — a portal triad, endothelial lining of a sinusoid, and the wavy collagen By the way, with PAS, I forgot to say the coagulum gives a faint pink color All these liver cells — I have not got time to show all the different stains — are heavily packed with glycogen

Figure 34 is an interesting case of a boy aged 11 months He shows an eosinophilic coagulum with only a few cells around this big vessel At the bottom right hand corner is a portal triad, and *from this extending from the space of Mall is an eosinophilic* coagulum going into the actual lobule And we believe that it is possibly going along the spaces of Disse or, as I shall demonstrate later, we think it alternatively may be going along the intralobular vessels, one of the arterioles which go halfway or two thirds of the way into the lobule The interesting thing about this case is that when you come a little bit lower down — a few more sections — you actually see fibrosis going on, and we feel that the collagenosis actually becomes fibrosis In other words, you are "reinforcing the concrete" The concrete is your collagen Later on you put in rein

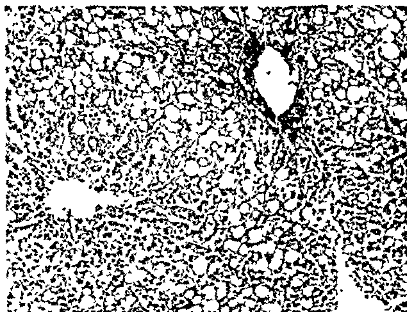


FIGURE 34 Liver from a child aged 3 yr 3 months showing fatty metamorphosis Note also (a) collagenous cuffing of vessel in upper right corner and (b) distension of Space of Mall of portal triad by serous exudate in lower left corner Modified Mallory connective tissue stain x 150

forcement which are *your* fibroblasts Now I will show you this area at a lower level

Figure 35 shows the coagulum extending from the space of Mall and it is being invaded by "streamer cells" which I think are probably fibroblasts

In Figure 36 you will see the other area around the vessel at a lower level with "streamer cells" invading eosinophilic coagulum

Figure 37 is a case which we *think* shows in the portal triads a serous exudation with an eosinophilic coagulum *and* an invasion of round cells If you remember Moschowitz (14) work, he thinks the round cells may be totipotential cells which become fibroblasts, endothelial cells, and so *on*

In Figure 38 you will see what we consider *is* an intralobular vessel This is right in the middle of the lobule And around the vessel is a sheath of eosinophilic coagulum which is becoming fibrosed When I do serial sections on the vessel I pick it up to

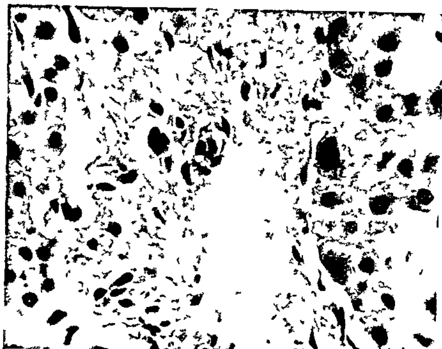
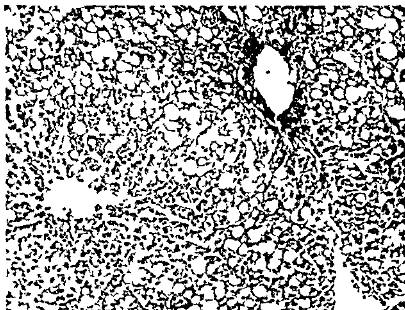


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FIGURE 32 Shows distension of a Space of Mall of a portal triad with serous exudate and deposition of eosinophilic coagulum Note the two bands of coagulum extending from the Space of Mall up into the lobule in the upper left hand corner *From liver of child aged 1 yr 3 months H & E x 600*

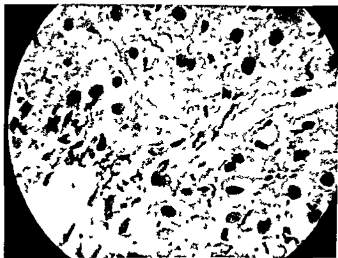


FIGURE 33 Shows a fibrillary band of eosinophilic coagulum extending from a portal triad (lower left hand corner) into the lobule diagonally along the perisinusoidal spaces This fibrillar relatively acellular coagulum gives all the tinctorial reactions of collagen From the liver of a child aged 4 months H & E x 400



FIGURE 34 Show ... the lobule from the Space of Mall in ... ascular deposition of eosinophilic coagu ... ntre From the liver of a child aged 1

about two thirds of the way towards the central lobular vein I trace it back – and unfortunately my block ends but it ends just before I get to the portal triad My presumption, though I cannot prove it, is that it was going to the portal triad This was an intra lobular vessel

And let me say one other thing in our cases I have come to the position that on histological examination I cannot tell what is a portal triad and what is not, because a lot of the intralobular vessels are clearly demarcated because of the surrounding eosinophilic co agulum which we think is within the vessel sheath Sometimes they have bile ducts within them which we see there is no distortion in the liver at all and sometimes one sees several portal triads together and apparently out of place And sometimes one sees the intralobular vessels and all that goes with them being clearly demarcated by this surrounding collagenosis

In Figure 39 you will see a fatty liver but the collagenosis is there around centrilobular vein And actually when you follow

FIGURE 52. Shows distension of a Space of Mall of a portal triad with sero ... ule and deposition of eosinophilic coagulum. Note the two bands of coagulum extending from the Space of Mall up into the lobule in the upper left hand corner. From liver of child aged 1 yr 6 months. H & E x 600

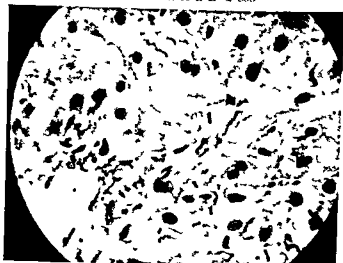


FIGURE 53. Shows a fibrillary band of eosinophilic coagulum extending from a portal triad (lower left hand corner) into the lobule diagonally along the perisinusoidal spaces. This fibrillar relatively acellular coagulum gives all the tinctorial reactions of collagen. From the liver of a child aged 4 months. H & E x 400.



FIGURE 34 Shows (a) eosinophilic coagulum extending into the lobule from the Space of Mall in the lower right hand corner and (b) perivascular deposition of eosinophilic coagulum around a large vessel to the left of centre From the liver of a child aged 11 months H & E x 150

about two thirds of the way towards the central lobular vein I *trace it back — and unfortunately my block ends, but it ends just* before I get to the portal triad My presumption, though I cannot prove it, is that it was going to the portal triad This was an intra lobular vessel

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In Figure 39 you will see a fatty liver, but the collagenosis is there, around centrilobular vein And actually when you follow

FIGURE 32 Shows distension of a Space of Mall of a portal triad with serous exudate and deposition of eosinophilic coagulum Note the two bands of coagulum extending from the Space of Mall up into the lobule in the upper left hand corner From liver of child aged 1 yr 3 months H & E x 600

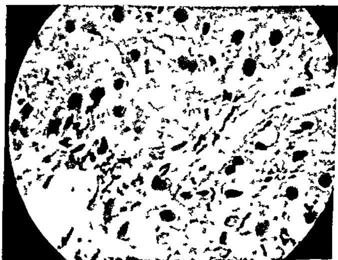


FIGURE 33 Shows a fibrillary band of eosinophilic coagulum extending from a portal triad (lower left hand corner) into the lobule diagonally along the perisinusoidal spaces This fibrillar relatively acellular coagulum gives all the tinctorial reactions of collagen From the liver of a child aged 4 months H & E x 400



FIGURE shows (a) eosinophilic coagulum extending into the lobule from the Space of Mall in the lower right hand corner and (b) [illegible] portion of eosinophilic coagulum around a large vessel to the left of centre. From the liver of a child aged 11 months. H & E × 150

about two thirds of the way towards the central lobular vein. I trace it back—*and unfortunately my block ends* but *it ends just* before I get to the portal triad. My presumption, though I cannot prove it, is that it was going to the portal triad. This was an intralobular vessel.

And let me say one other thing: in our cases I have come to the position that on histological examination I cannot tell what is a portal triad and what is not, because a lot of the intralobular vessels are clearly demarcated because of the surrounding eosinophilic coagulum which we think is within the vessel sheath. Sometimes they have bile ducts *within them which we see; there is no distortion* in the liver at all, and sometimes one sees several portal triads together and apparently out of place. And sometimes one sees the intralobular vessels and all that goes with them being clearly demarcated by this surrounding collagenosis.

In Figure 39 you will see a fatty liver, but the collagenosis is there around centrilobular vein. And actually when you fu

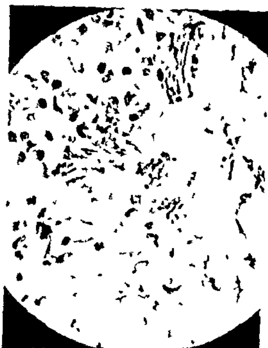


FIGURE 35 Serial section of Figure 34 about 100 μ deeper high power of area (a) Figure 34 showing "streamer cells" (fibroblasts) invading eosinophilic coagulum which is extending into the lobule from the Space of Mall. H & E x 400

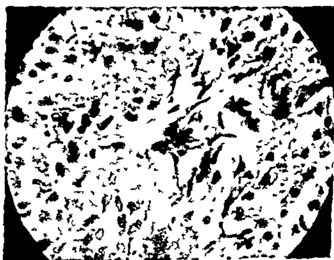


FIGURE 36 Serial section of Figure 34 about 100 μ deeper high power of area (b) Figure 34 showing "streamer cells" (fibroblasts) invading perivascular eosinophilic coagulum *H & E x 400*

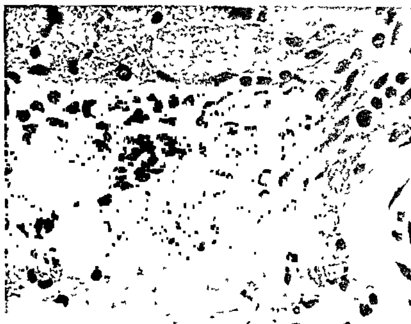


FIGURE 37 Shows distension of space of Mall with serous exudate there is also eosinophilic coagulum present which is being invaded by small round cells From liver of child aged 2½ yrs H & E x 600

the vessel in serial section, from the portal triad, it only goes up two thirds of the way into the lobule In other cases you can see the fibrosis around the central vein and you can see the connections between them and the portal triad But this is a fatty liver with an underlying pathology, which we have described and which we think is "collagenosis"

In Figure 40 you will see some of the end results a central lobular vein connecting with the portal triads which are showing some cellular activity And once this seems to start, away she goes, so that finally you get, in the next picture (Figure 41) this sort of thing cuffing, reticulinosis, eosinophilic collagenosis, and marked unilobular fibrosis, until finally (Figure 42) you get the classical picture which we have in Jamaica of this hepatic fibrosis I don't like the term 'cirrhosis' You see the centrilobular vein heavily cuffed, you see the portal triads and their interconnections There is also a nodular reconstruction, if I can use Popper's(12) terminology here And that is the final condition which started as

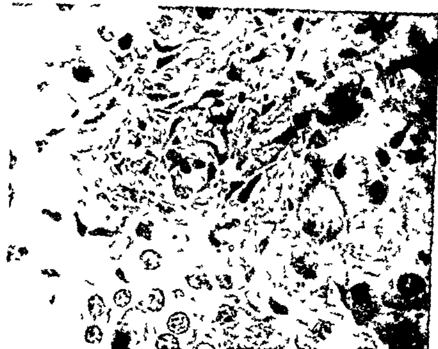


Figure 38 Shows an intralobular vessel demarcated by sheath containing eosinophilic coagulum From liver of child aged 1 yr 6 months H & E x 600

serous exudation followed by collagenosis followed by fibrosis during repeated attacks and you find all this taking place at the same time 25 per cent of our cases had serous exudation 80 per cent had collagenosis 30 per cent had gross fibrosis but 75 per cent had mild or severe portal tract activity

So far I have never mentioned anything about liver cell damage I have to be very careful here I have been influenced by Dr Popper's(12) work and I have tried to classify liver cell damage according to 1 plus 2 plus and 3 plus the way he did and quite frankly I was unable to do it It is interesting that significant liver damage the 2 to 3 plus that Popper describes I get in 25 per cent of cases we get marked fibrosis in 30 per cent and actually these cases overlap and are the same

So in the early cases I did not see any liver cell damage One of the reasons why I have not enlarged on liver cell damage is that the appearances of the hepatic cells varied with the fixative used—we used six in all in one or two test cases However the appearance of the eosinophilic coagulum never varied with the fixative

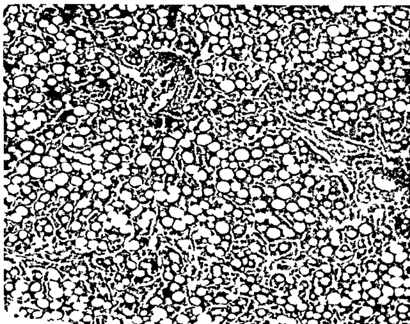


FIGURE 39 Shows fatty metamorphosis, collagen cuffing of centrilobular vein and fibrotic sheathing of intralobular vessel. From liver of a child aged 10 months. H & E × 150

Liver cell damage is nil at this moment in the investigation except where you have gross hepatic cirrhosis.

Watson: You don't see much monocellular exudate such as one sees in a cirrhosis in the portal spaces?

Hill: No.

Campbell: Does that serous exudate which you described in about 25 per cent of your cases look like the serous exudate that Rossle(15) and Eppinger, Kaunitz and Popper(16) have described in adult livers — their "serous hepatitis"? Is it related do you think to general edema? Was there any correlation between the incidence of this serous exudate and general edema in your patients?

Hill: None of my patients had general edema except the five with fatty livers. They all had ascites.

As regards Rossle's(17) work, it is rather interesting that we became aware of it when we had finished our survey of these slides.

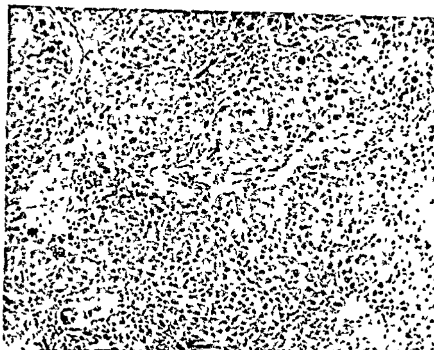


FIGURE 40 Shows hepatic fibrosis with marked cellularity of portal triads and connecting fibrous bands between portal tracts and centrilobular vein From liver of child aged 2 yrs H & E × 150

Rossle described this, and Eppinger(16) took it up but subsequently it was rather condemned by Keschner and Klemperer(18) Rossle did not go as far as we did, but his general reasoning was similar to ours

Campbell He did not postulate that it went on to collagenosis?

Hill No we went further

Popper He had assumed that the serous exudate is transformed to fibers

Hill He did in his hypothesis

Turner Did you do any stains for polysaccharides?

Hill As periodic acid Schiff

Turner Does it represent anything unique?

Gyorgy Negative

Neefe Are the lymph nodes enlarged in these cases?

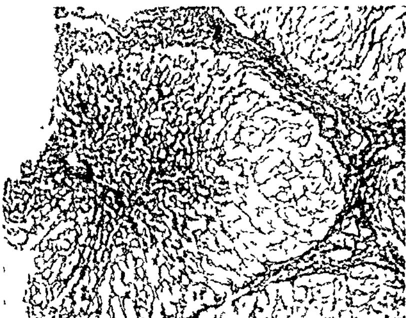


FIGURE 41 Shows hepatic fibrosis with centrilobular cuffing perisinusoidal and perilobular reticulinosis From liver of a child aged 4 yrs Modified Foot Wilder reticulin stain x 150

Hill We have superficial adenopathy, but scabies is very common

Neefe But around the bile ducts and omentum you don't have marked adenopathy?

Hill No, but I must say – I have not mentioned this – I think I am beginning to see that there is some dilation of the perivascular lymphatics (with endothelial lining) Whether there is any relationship between that and the high protein content of ascitic fluid we do not know at this moment

Silliphant Is cirrhosis a very prevalent disease among the adults in Jamaica?

Hill Well, I think it is Waterlow(3) in his survey s d 10 p cent of all the children he examined had enlarged li

Silliphant And may I ask what was your techniq in these 60 cases?

Hill Silverman needle

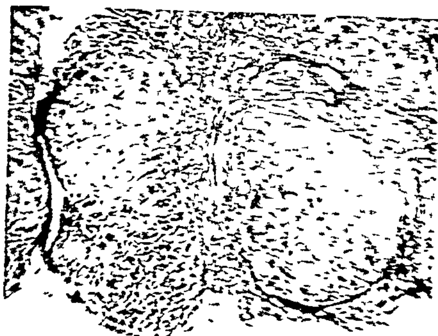


FIGURE 42. Shows marked hepatic fibrosis with ... From liver of child aged ...

Sherlock How do your results compare with those of ... C. Waterlow? I thought he ... change in the livers of West Indian infants.

Hill That is what I said.

Sherlock Why the difference? ... observations were made in the same place.

Gyorgy That is a very good question.

Hill Well may I say th... months The figure for the Jamaicans and that was ... more extensive

Sherlock You never saw

Hill In 5 cases

Sherlock In the early sta...

Hill Our youngest case ... youngest case if you want to

months We can show clinically that they are quite different They have general nutritional edema and show these deficiencies, and they do not want to eat But yet they have this underlying pathology

Watson Your thought is that the fatty liver is purely coincidental?

Hill Yes

Watson That they are two unrelated processes in all likelihood?

Hill Yes

Best I think we should have Dr Gyorgy s comments

Gyorgy I first want to thank Dr Hill and Dr Taylor Principal of the University College of the West Indies (Jamaica) for giving our group at the University of Pennsylvania the privilege of observing this fascinating work and of inviting us to be of some assistance It was a thrilling experience and a clear unmistaken example of serendipity We went down to Jamaica thinking that we were going to find fatty liver based on low protein intake Last year you will remember, in our discussion to Dr Davies presentation we generalized and came to the conclusion that all over the tropical belt we are dealing with low protein diet which – in analogy to animal experiments – should lead to fatty liver This fatty liver disease has been reported from various parts of the world And then in Jamaica we did find low protein intake but no or only as an exception, fatty liver This is a fact, confirmed also by my own personal observation

Dr Rhodes, who is a very astute young clinician and who deserves all credit for the clinical work – I am glad Professor Hill concurs with me therein –, made these observations Her clinical classification is first class

In the light of our observations in Jamaica we would have to modify our concept regarding forms of hepatic injury During the past 12 years we have forgotten serous inflammation as a manifestation of hepatic injury On the basis of animal experiments, we distinguished two classes of liver injury One was fatty liver with cirrhosis and the other one was acute or subacute necrosis focal and zonal, central or massive and we have not given any position to serous inflammation

Now, this serous inflammation, as Professor Hill has clearly demonstrated, exists in clinical material For the present we are unable to give any etiological foundation for this very widespread

incidence of the serous inflammation, with its sequelae I am impressed — and I have to thank Dr Popper for calling my attention to it — by Eppinger's work on experimental production of serous inflammation(16) by allylformate and *similar compounds in animals* We have now to resume this work of Eppinger and Popper, and see whether we could reproduce it in animals, perhaps easier on a dietary basis Protein deficiency, which is a regular etiological factor in liver disease in Jamaica, should act as such dietary background

In fatty cirrhosis we have only deficiency of choline or its precursors (methionine in proteins) In massive necrosis and post necrotic scarring we have a combination of dietary deficiency and of toxic components (intestinal flora, infection?) It is my guess that in Jamaica we will find the same combination of dietary deficiency and of some toxic factors

The fact that we encounter this disease in Jamaica in very young infants and that practically all young infants have this disease leads me to believe that we may deal with an endemic infection Young infants acquire the disease and either die or survive In older children or adults the disease is practically unknown in its acute form One and a half years ago I saw a 3 year old colored child in Jamaica with massive ascites, large, hard liver One year later the same child lost its ascites, the liver became not palpable and the general condition was very much improved

In conclusion I repeat, it is my personal feeling that the prevalent liver disease in young Jamaican children is based on the interaction of infection or toxic factors and dietary (protein?) deficiency Obviously this is a third class of liver injury and we have to thank Professor Hill and his colleagues as well as the Jamaican children for making this new class a reality

Popper This takes me back to work which I did in Eppinger's clinic many years ago on experimental production as well as on clinical and pathological features of serous hepatitis The first question *is one of nomenclature* Can we call the lesion an inflammation? Eppinger(16) and Rossle(17) applied the term "serous inflammation" For years this question has been discussed in Germany and Austria and I personally feel that Klemperer(18) is right in speaking of "toxic edema" rather than of "*inflammation*" I am now not happy about the term "inflammation" although in the original papers, for a few of which I was responsible myself(19, 20), we spoke of "serous hepatitis"

The next question concerns the origin of the proteinic material in the Disse spaces or in the Mall spaces We can see in human pathology a considerable amount of it, for instance, in congestion and especially, as Rossle(15) pointed out, in hyperthyroidism In this latter condition we may see a characteristic centrolobular fibrosis as Dr Smetana will confirm One could use Dr Hill's term of "fibrotic cuff" Is this proteinic material serum protein which has escaped from the sinusoids and which is being transformed as Eppinger and Rossle claim into collagenous tissue? There is some evidence for formation of fibers independent of cells It has been shown that in tissue cultures away from cells fibers may form out of protein However in my opinion this problem has never been completely solved Dr Hill I would like to know whether or not you consider the protein in the Disse spaces in your preparations serum protein which has escaped the capillaries?

While working with Eppinger I tried under the direction of Hutinger to develop a fluorescence microscopic method (this was my first contact with fluorescence microscopy) for the differentiation of serum and tissue protein We were hoping that specific fluorescence after staining with fluorescent dyes would reveal the origin of the protein precipitate which is seen in the Disse and Mall spaces and which you have just described in your specimens I was never convinced that the method as we described it(21) (22) is really specific However Eppinger in his latest book which represents a new and enlarged edition(23) of the previous book *Serous Inflammation*(16) brings many pictures utilizing a modification of our fluorescence microscopic method to show serum protein in the Disse spaces and in the interstitial tissue spaces It is rather gratifying that due to Dr Hill's efforts these old studies are now coming to life again though I would personally prefer the term of "toxic edema"

I have one more question Due to the kindness of Dr Gyorgy, I was privileged to see some of the slides which Dr Hill has just discussed If I remember correctly there was at least in the early cases, rather marked centrolobular congestion This of course could easily explain the escape of a large amount of serum protein into the Dissé spaces We see such an escape of protein quite typically in passive congestion In long standing congestion fibrotic bands or septa extend from the central zones and sometimes also from the portal canals similar to those you have demonstrated To what degree do you feel is congestion responsible for the presented

lesion if we assume that the protein in the Dissé spaces originates from the serum?

Hill I feel that the origin is from the blood

As regards congestion, I simply don't know. These livers are congested Why, we simply don't know When we do stains, we never see evidence of fibrin, red cells, or anything else It is just protein precipitate after we have precipitated it with our fixative I have described it as an exudate That does not say that it is an inflammation if you look up the correct definition of an exudate That is a histologist's description

Best Any general discussion?

Sherlock I did not know we had gone back to the term "serous hepatitis," and thought most of the findings we ascribed as being due to serous hepatitis were autopsy changes Certainly this opening of perisinous spaces was described by you fairly recently as occurring as an autopsy change

Popper Yes

Sherlock Are we going back to serous hepatitis?

Popper I believe we are dealing with a problem of gradation The sinusoids of the liver are readily permeable for serum protein That means, serum protein may be present in the Dissé spaces, especially in the human as a result of slight functional alterations These serum proteins may be drained away by the lymphatics If excessive amounts escape, i e , more than the lymphatics can handle, the escaped serum proteins accumulate, widen the Dissé spaces and produce edema This edema or serous hepatitis impairs the exchange between the capillary and the parenchymal cells, thus eventually damaging the cells Due to the increased permeability, toxic material including bacteria may escape the sinusoids and produce lesions As additional sequela of a chronic alteration, fiber formation may result either from the precipitate itself or stimulated by the precipitate This is possibly related to the appearance of polychromatic mucopolysaccharides in the interstitial tissue(24) Whether we have the right to call this process collagenosis or not, I really don't know but I think Dr Hill himself asked that quotation marks be put on the word "collagen" Increased permeability for protein may be produced rapidly by anoxia and in the human, in contrast to most animals the right heart failure during the agonal period suffices to produce an acute edema Therefore, we do see in human autopsy

material except in instantaneous death e g due to a crash almost invariably the Disee spaces dilated and filled with proteinic precipitate whereas we do not see this in biopsy specimens(25) In Eppinger s laboratory we noted wide Dissé spaces in apparently normal persons who had been executed by hanging I have seen it in persons who died from illuminating gas intoxication or acute cardiac failure This means that a few minutes of anoxia in the agonal period under such circumstances suffice to open up the Disse spaces We wrongly called these changes serous inflammation I am now so strongly opposed to this name because I believe in our studies with Eppinger we overlooked this agonal change However there are more severe degrees of increased permeability which may occur during life Morphologically they are recognized by the formation of a thick coagulum which is precipitated by the process of fixation in the histologic preparation Such firm and thick coagula usually associated with very wide tissue spaces cannot be considered an agonal process and point to longer standing intravital changes We have observed such lesions in passive congestion in infections and intoxications among other conditions Dr Hill has shown them in his specimens I believe therefore that this represents a more severe degree of lesion the milder degree of which may be produced by agonal processes

I believe that Dr Hill is dealing with the effect of a capillary toxin plus congestion The combination of these two factors may lead to a massive escape of serum protein into the Disse spaces with subsequent coagulation of this tissue protein and production of the described cuffs finally producing the end stage which he has so clearly shown The only point I was arguing was the location of the block he was referring to which was not quite clear to me in the beginning I believe that the lesion starts with damage to the sinusoids

Best What causes the congestion?

Hill I don t know

Gyorgy Clinically there is absolutely no reason to assume there is a congestion

Watson It does not look like an ordinary passive congestion

Hill No it is patchy

Gyorgy The subjects are perfectly normal looking children walking around

Popper I would like to correct myself I did not mean congestion in the sense of an obstruction to the return of blood to the heart Maybe we can call it stasis If a morphologist sees a dilated vessel he speaks of *congestion though it could very well be stasis*

Watson Active congestion would be another term

Gyorgy W Gerlach described in the well known *Handbook* of Henke Lubarsch(26) a condition called tropical liver which is characterized by active congestion

Hill If you want any information go back to the German literature of thirty or more years ago

Gyorgy And the cause of it is unknown

Sherlock Could some of the hepatic changes be related to beriberi? Perhaps due to the high output heart failure which is known to occur with beriberi

It has nothing to do with a B 1 lack?

Hill As far as vitamin deficiencies they are not very prevalent in Jamaica I quote people like Waterlow

Popper In Eppinger's laboratory I examined beriberi livers(16) We noted stasis or congestion whichever you care to call it as well as perisinusoidal fluid accumulation not only in the central part of the lobule but all the way from the central zone to the portal canal In Dr Hill's preparation it was mainly in the centrolobular zone I also believe that the proteinic material in the Dissé spaces was less dense in beriberi By the way Rossle(27) described similar changes *in rheumatic fever an endophlebitis* and *periphlebitis* which he explained similarly on the escape of serum protein into the Dissé spaces The lesion resembles somewhat the one Dr Hill has shown

Watson I judge that these patients did not have any neuropathy did they Dr Hill?

Hill The answer *is no but neuropathies* are fairly common among the adults in Jamaica

Gyorgy Unexpectedly even *hypertension and eclampsia* are also common

Watson Any changes in the legs pain and reflex changes?

Neefe Do they ever become visibly jaundiced?

Hill Only terminally with massive hepatic fibrosis if I may use that term

Popper Serous hepatitis as we saw it was not necessarily associated with jaundice except in late stages Sometimes jaundice is absent even in the presence of a severe fibrosis

Hill Why do you think there are the wide open spaces in the sinusoids? Sometimes several of them as I showed were demarcated by the collagen but sometimes there does not seem to be any Rossle describes it too Why?

Popper Rossle spoke of a plasma flow That means wide open sinusoids which contain only a few red cells If I remember right I saw that in your specimens but it did not occur to me until now Is that right?

Hill Yes

Popper I do not remember the explanation which Rossle gave for this plasma flow I think the morphogenesis of the lesion presented by you can be most easily explained (accepting the correction which you just made) by a capillary paralysis with dilation of the sinusoids and increased permeability of the sinusoidal wall for protein Whether this is due to nutritional factors is of course another problem not directly related to the morphogenesis

Turner Sudden death in this group is not one of the features?

Hill No

Dauphinee Dr Hill you mentioned that the serum albumin in these patients tended to be on the low side of normal

Hill Yes

Dauphinee Was there any difference in the level of the serum albumin between those patients who had the large fatty liver with generalized edema those with a large liver but no peripheral edema and those who had a definite fibrosis in their liver or have you enough information to say?

Hill No the serum albumin was below 3 5 in 80 per cent of our cases and they were scattered

Dauphinee Did these include the 5 per cent who were grossly edematous?

Hill Yes

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